EXHIBIT A

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Lau et al.

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(54) **ACYLATED GLP-1 COMPOUNDS**

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PCT Pub. Date: **Sep. 21, 2006**

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(30) **Foreign Application Priority Data**

Mar. 18, 2005 (EP) 05102171

(51) **Int. Cl.**

A61K 38/26	(2006.01)
A61P 3/10	(2006.01)
A61P 7/12	(2006.01)
C07K 14/605	(2006.01)

(52) **U.S. Cl.** **514/11.7**

(58) **Field of Classification Search** ..... None
See application file for complete search history.

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(57) **ABSTRACT**

Protracted GLP-1 compounds and therapeutic uses thereof.

6 Claims, No Drawings

ACYLATED GLP-1 COMPOUNDS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a 35 U.S.C. §371 national stage application of International Patent Application PCT/EP2006/060855 (published as WO 2006/097537), filed Mar. 20, 2006, which claimed priority of European Patent Application 05102171.5, filed Mar. 18, 2005; this application further claims priority under 35 U.S.C. §119 of U.S. Provisional Application 60/664,497, filed Mar. 23, 2005.

FIELD OF THE INVENTION

This invention relates to the field of therapeutic peptides, i.e. to new protracted GLP-1 compounds.

BACKGROUND OF THE INVENTION

A range of different approaches have been used for modifying the structure of glucagon-like peptide 1 (GLP-1) compounds in order to provide a longer duration of action in vivo.

WO 96/29342 discloses peptide hormone derivatives wherein the parent peptide hormone has been modified by introducing a lipophilic substituent in the C-terminal amino acid residue or in the N-terminal amino acid residue.

WO 98/08871 discloses GLP-1 derivatives wherein at least one amino acid residue of the parent peptide has a lipophilic substituent attached.

WO 99/43708 discloses GLP-1(7-35) and GLP-1(7-36) derivatives which have a lipophilic substituent attached to the C-terminal amino acid residue.

WO 00/34331 discloses acylated GLP-1 analogs.

WO 00/69911 discloses activated insulinotropic peptides to be injected into patients where they are supposed to react with blood components to form conjugates and thereby alledgedly providing longer duration of action in vivo.

WO 02/46227 discloses GLP-1 and exendin-4 analogs fused to human serum albumin in order to extend in vivo half-life.

Many diabetes patients particularly in the type 2 diabetes segment are subject to so-called "needle-phobia", i.e. a substantial fear of injecting themselves. In the type 2 diabetes segment most patients are treated with oral hypoglycaemic agents, and since GLP-1 compounds are expected to be the first injectable product these patients will be administered, the fear of injections may become a serious obstacle for the widespread use of the clinically very promising GLP-1 compounds. Thus, there is a need to develop new GLP-1 compounds which can be administered less than once daily, e.g. once every second or third day preferably once weekly, while retaining an acceptable clinical profile.

SUMMARY OF THE INVENTION

The invention provides a GLP-1 analog having a modification of at least one non-proteogenic amino acid residue in positions 7 and/or 8 relative to the sequence GLP-1(7-37) (SEQ ID No 1), which is acylated with a moiety to the lysine residue in position 26, and where said moiety comprises at least two acidic groups, wherein one acidic group is attached terminally.

The present invention also provides pharmaceutical compositions comprising a compound according to the present invention and the use of compounds according to the present invention for preparing medicaments for treating disease.

The invention provides a method for increasing the time of action in a patient of a GLP-1 analog, characterised in acylating said GLP-1 analog with a moiety B—U' as disclosed in any of the preceding claims, on the lysine residue in position 26 of said GLP-1 analog.

DESCRIPTION OF THE INVENTION

In the present specification, the following terms have the indicated meaning:

The term "polypeptide" and "peptide" as used herein means a compound composed of at least five constituent amino acids connected by peptide bonds. The constituent amino acids may be from the group of the amino acids encoded by the genetic code and they may be natural amino acids which are not encoded by the genetic code, as well as synthetic amino acids. Natural amino acids which are not encoded by the genetic code are e.g., γ-carboxyglutamate, ornithine, phosphoserine, D-alanine and D-glutamine. Synthetic amino acids comprise amino acids manufactured by chemical synthesis, i.e. D-isomers of the amino acids encoded by the genetic code such as D-alanine and D-leucine, Aib (α-aminoisobutyric acid), Abu (α-aminobutyric acid), Tle (tert-butylglycine), β-alanine, 3-aminomethyl benzoic acid, anthranilic acid.

The 22 proteogenic amino acids are:

Alanine, Arginine, Asparagine, Aspartic acid, Cysteine, Cystine, Glutamine, Glutamic acid, Glycine, Histidine, Hydroxyproline, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, Valine.

Thus a non-proteogenic amino acid is a moiety which can be incorporated into a peptide via peptide bonds but is not a proteogenic amino acid. Examples are γ-carboxyglutamate, ornithine, phosphoserine, the D-amino acids such as D-alanine and D-glutamine, Synthetic non-proteogenic amino acids comprise amino acids manufactured by chemical synthesis, i.e. D-isomers of the amino acids encoded by the genetic code such as D-alanine and D-leucine, Aib (α-aminoisobutyric acid), Abu (α-aminobutyric acid), Tle (tert-butylglycine), 3-aminomethyl benzoic acid, anthranilic acid, des-amino-Histidine, the beta analogs of amino acids such as β-alanine etc. D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, N^{α}-acetyl-histidine, α-fluoromethyl-histidine, α-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid;

The term "analogue" as used herein referring to a polypeptide means a modified peptide wherein one or more amino acid residues of the peptide have been substituted by other amino acid residues and/or wherein one or more amino acid residues have been deleted from the peptide and/or wherein one or more amino acid residues have been deleted from the peptide and or wherein one or more amino acid residues have been added to the peptide. Such addition or deletion of amino acid residues can take place at the N-terminal of the peptide and/or at the C-terminal of the peptide. A simple system is often used to describe analogues: For example [Arg^{34}]GLP-1(7-37)Lys designates a GLP-1(7-37) analogue wherein the naturally occurring lysine at position 34 has been substituted with arginine and wherein a lysine has been added to the terminal amino acid residue, i.e. to the Gly^{37}. All amino acids for which the optical isomer is not stated is to be understood to mean the L-isomer. In embodiments of the invention a

maximum of 17 amino acids have been modified. In embodiments of the invention a maximum of 15 amino acids have been modified. In embodiments of the invention a maximum of 10 amino acids have been modified. In embodiments of the invention a maximum of 8 amino acids have been modified. In embodiments of the invention a maximum of 7 amino acids have been modified. In embodiments of the invention a maximum of 6 amino acids have been modified. In embodiments of the invention a maximum of 5 amino acids have been modified. In embodiments of the invention a maximum of 4 amino acids have been modified. In embodiments of the invention a maximum of 3 amino acids have been modified. In embodiments of the invention a maximum of 2 amino acids have been modified. In embodiments of the invention 1 amino acid has been modified.

The term "derivative" as used herein in relation to a peptide means a chemically modified peptide or an analogue thereof, wherein at least one substituent is not present in the unmodified peptide or an analogue thereof, i.e. a peptide which has been covalently modified. Typical modifications are amides, carbohydrates, alkyl groups, acyl groups, esters and the like. An example of a derivative of GLP-1(7-37) is $N^{\epsilon 26}$-((4S)-4-(hexadecanoylamino)-carboxy-butanoyl)[Arg^{34}, Lys^{26}] GLP-1-(7-37).

The term "GLP-1 peptide" as used herein means GLP-1(7-37) (SEQ ID No 1), a GLP-1(7-37) analogue, a GLP-1(7-37) derivative or a derivative of a GLP-1(7-37) analogue. In one embodiment the GLP-1 peptide is an insulinotropic agent.

The term "insulinotropic agent" as used herein means a compound which is an agonist of the human GLP-1 receptor, i.e. a compound which stimulates the formation of cAMP in a suitable medium containing the human GLP-1 receptor (one such medium disclosed below). The potency of an insulinotropic agent is determined by calculating the EC_{50} value from the dose-response curve as described below.

Baby hamster kidney (BHK) cells expressing the cloned human GLP-1 receptor (BHK-467-12A) were grown in DMEM media with the addition of 100 IU/mL penicillin, 100 μg/mL streptomycin, 5% fetal calf serum and 0.5 mg/mL Geneticin G-418 (Life Technologies). The cells were washed twice in phosphate buffered saline and harvested with Versene. Plasma membranes were prepared from the cells by homogenisation with an Ultraturrax in buffer 1 (20 mM HEPES-Na, 10 mM EDTA, pH 7.4). The homogenate was centrifuged at 48,000×g for 15 min at 4° C. The pellet was suspended by homogenization in buffer 2 (20 mM HEPES-Na, 0.1 mM EDTA, pH 7.4), then centrifuged at 48,000×g for 15 min at 4° C. The washing procedure was repeated one more time. The final pellet was suspended in buffer 2 and used immediately for assays or stored at −80° C.

The functional receptor assay was carried out by measuring cyclic AMP (cAMP) as a response to stimulation by the insulinotropic agent. cAMP formed was quantified by the AlphaScreen™ cAMP Kit (Perkin Elmer Life Sciences). Incubations were carried out in half-area 96-well microtiter plates in a total volume of 50 μL buffer 3 (50 mM Tris-HCl, 5 mM HEPES, 10 mM $MgCl_2$, pH 7.4) and with the following addiditions: 1 mM ATP, 1 μM GTP, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 0.01% Tween-20, 0.1% BSA, 6 μg membrane preparation, 15 μg/mL acceptor beads, 20 μg/mL donor beads preincubated with 6 nM biotinyl-cAMP. Compounds to be tested for agonist activity were dissolved and diluted in buffer 3. GTP was freshly prepared for each experiment. The plate was incubated in the dark with slow agitation for three hours at room temperature followed by counting in the Fusion™ instrument (Perkin Elmer Life Sciences). Concentration-response curves were plotted for the individual compounds and EC_{50} values estimated using a four-parameter logistic model with Prism v. 4.0 (GraphPad, Carlsbad, Calif.).

The term "DPP-IV protected" as used herein referring to a polypeptide means a polypeptide which has been chemically modified in order to render said compound resistant to the plasma peptidase dipeptidyl aminopeptidase-4 (DPP-IV). The DPP-IV enzyme in plasma is known to be involved in the degradation of several peptide hormones, e.g. GLP-1, GLP-2, Exendin-4 etc. Thus, a considerable effort is being made to develop analogues and derivatives of the polypeptides susceptible to DPP-IV mediated hydrolysis in order to reduce the rate of degradation by DPP-IV. In one embodiment a DPP-IV protected peptide is more resistant to DPP-IV than GLP-1(7-37) or Exendin-4(1-39).

Resistance of a peptide to degradation by dipeptidyl aminopeptidase IV is determined by the following degradation assay:

Aliquots of the peptide (5 nmol) are incubated at 37° C. with 1 μL of purified dipeptidyl aminopeptidase IV corresponding to an enzymatic activity of 5 mU for 10-180 minutes in 100 μL of 0.1 M triethylamine-HCl buffer, pH 7.4. Enzymatic reactions are terminated by the addition of 5 μL of 10% trifluoroacetic acid, and the peptide degradation products are separated and quantified using HPLC analysis. One method for performing this analysis is: The mixtures are applied onto a Vydac C18 widepore (30 nm pores, 5 μm particles) 250×4.6 mm column and eluted at a flow rate of 1 ml/min with linear stepwise gradients of acetonitrile in 0.1% trifluoroacetic acid (0% acetonitrile for 3 min, 0-24% acetonitrile for 17 min, 24-48% acetonitrile for 1 min) according to Siegel et al., Regul. Pept. 1999; 79:93-102 and Mentlein et al. Eur. J. Biochem. 1993; 214:829-35. Peptides and their degradation products may be monitored by their absorbance at 220 nm (peptide bonds) or 280 nm (aromatic amino acids), and are quantified by integration of their peak areas related to those of standards. The rate of hydrolysis of a peptide by dipeptidyl aminopeptidase IV is estimated at incubation times which result in less than 10% of the peptide being hydrolysed.

The term "C_{1-6}-alkyl" as used herein means a saturated, branched, straight or cyclic hydrocarbon group having from 1 to 6 carbon atoms. Representative examples include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, tert-pentyl, n-hexyl, isohexyl, cyclohexane and the like.

The term "pharmaceutically acceptable" as used herein means suited for normal pharmaceutical applications, i.e. giving rise to no adverse events in patients etc.

The term "excipient" as used herein means the chemical compounds which are normally added to pharmaceutical compositions, e.g. buffers, tonicity agents, preservatives and the like.

The term "effective amount" as used herein means a dosage which is sufficient to be effective for the treatment of the patient compared with no treatment.

The term "pharmaceutical composition" as used herein means a product comprising an active compound or a salt thereof together with pharmaceutical excipients such as buffer, preservative, and optionally a tonicity modifier and/or a stabilizer. Thus a pharmaceutical composition is also known in the art as a pharmaceutical formulation.

The term "treatment of a disease" as used herein means the management and care of a patient having developed the disease, condition or disorder. The purpose of treatment is to combat the disease, condition or disorder. Treatment includes the administration of the active compounds to eliminate or

control the disease, condition or disorder as well as to alleviate the symptoms or complications associated with the disease, condition or disorder.

In another aspect the present invention relates to an acylated GLP-1 analogue that can bind to albumin and the GLP-1 receptor simultaneously.

In another aspect the present invention relates to an acylated GLP-1 analogue that bind to the GLP-1 receptor with an affinity below 100 nM, preferable below 30 nM in the presence of 2% albumin.

In another aspect the present invention relates to an acylated GLP-1 analogue which affinity to the GLP-1 receptor is only partly decreased when comparing the affinity in the presence of very low concentration (e.g. 0.005% to 0.2%) of human albumin to the affinity in the presence of 2% human albumin. The shift in binding affinity under these conditions is less than 50 fold, preferable below 30 fold and more preferable below 10 fold.

The term "albumin binding moiety" as used herein means a residue which binds non-covalently to human serum albumin. The albumin binding residue attached to the therapeutic polypeptide typically has an affinity below 10 μM to human serum albumin and preferably below 1 μM. A range of albumin binding residues are known among linear and branched lipohophillic moieties containing 4-40 carbon atoms having a distal acidic group.

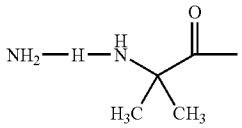
The term "hydrophilic linker" as used herein means a spacer that separates a peptide and an albumin binding residue with a chemical moiety which comprises at least 5 non-hydrogen atoms where 30-50% of these are either N or O.

The term "acidic groups" as used herein means organic chemical groups which are fully or partly negatively charged at physiological pH. The pKa value of such groups is below 7, preferable below 5. This includes but is not limited to carboxylic acids, sulphonic acids, phosphoric acids or heterocyclic ring systems which are fully or partly negatively charged at physiological pH.

In the below structural formula II the moiety U is a diradical may be attached to the terminal groups B and the aminogroup of the lysine amino acid in the peptide in two different ways. In embodiments of the invention the U in formula II is attached with the group B attached at the end of the alkyl chain and the peptide at the other end.

In the formulas below the terminal bonds from the attached groups are to be regarded as attachment bonds and not ending in methylene groups unless stated.

In the formulas below



means the H_2N-His-Aib-N-terminal of the GLP-1 analogue.

In an embodiment the invention provides a GLP-1 analog acylated with a lipophilic albumin binding moiety containing at least two free acidic chemical groups attached via a non natural amino acid linker to the lysine residue in position 26.

In an embodiment, the term free acidic chemical groups is to be understood as having the same meaning as "acidic groups" as used herein.

In an embodiment the invention provides an acylated GLP-1 analog where said GLP-1 analog is stabilised against DPP-IV by modification of at least one amino acid residue in positions 7 and 8 relative to the sequence GLP-1(7-37) (SEQ ID No 1), and where said acylation is a diacid attached to the lysine residue in position 26 optionally via a non natural amino acid hydrophilic linker.

In an embodiment of the invention a GLP-1 analog having a modification of at least one non-proteogenic amino acid residue in positions 7 and/or 8 relative to the sequence GLP-1(7-37) (SEQ ID No 1), which is acylated with a moiety to the lysine residue in position 26, and where said moiety comprises at least two acidic groups, wherein one acidic group is attached terminally.

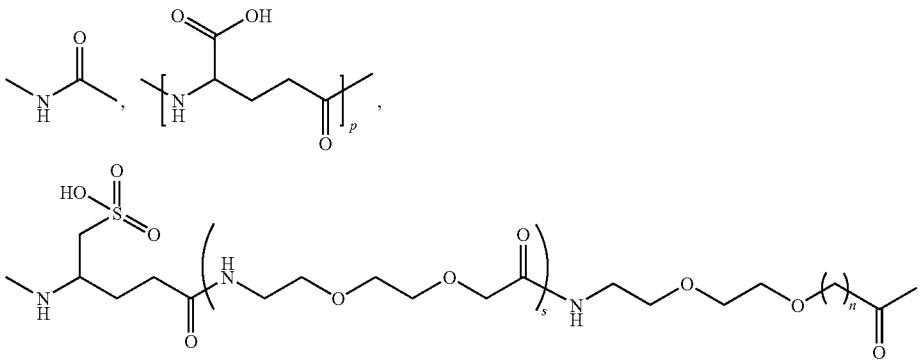
An embodiment provides a GLP-1 analog according to the above embodiment, wherein the moiety attached in position 26 comprises a hydrophilic linker.

An embodiment provides a GLP-1 analog according to the above embodiments, wherein the hydrophilic linker comprises at least 5 non-hydrogen atoms where 30-50% of these are either N or O.

An embodiment provides a GLP-1 analog according to any of the above embodiments, wherein the moiety attached in position 26 comprises an albumin binding moiety separated from the peptide by the hydrophilic linker.

An embodiment provides a GLP-1 analog according to the above embodiment, wherein the albumin binding moiety is a linear or branched lipophilic moiety containing 4-40 carbon atoms having a distal acidic group.

An embodiment provides a GLP-1 analog according to any of the above embodiments, wherein the acylated moiety is B—U', where U' is selected from



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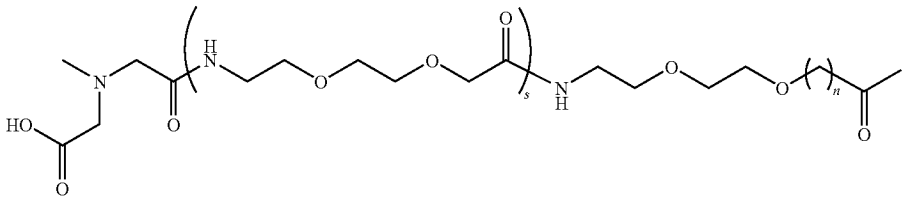
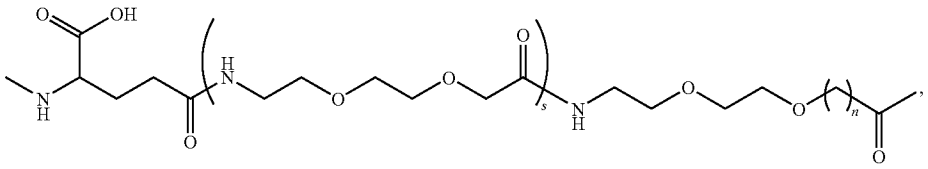
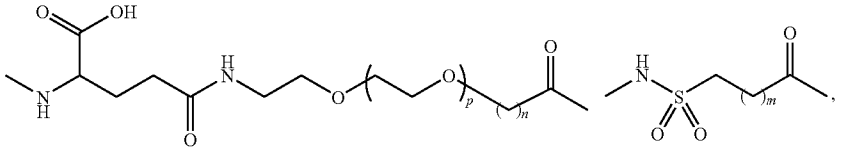
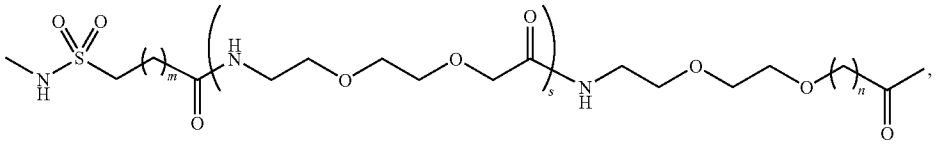
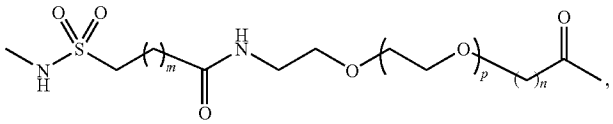
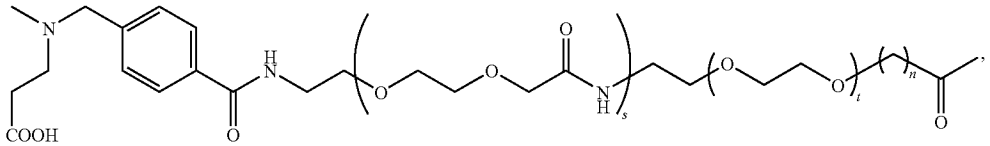


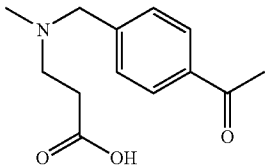


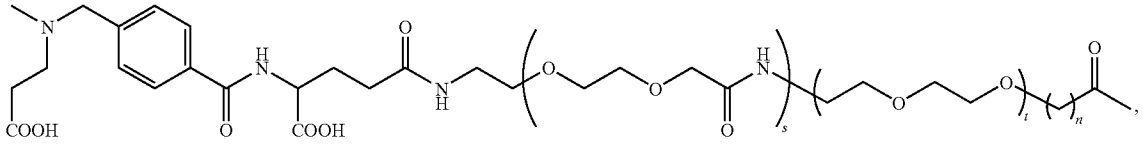




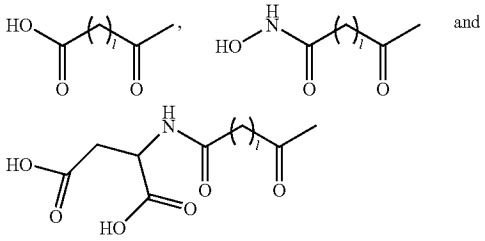








m is 0, 1, 2, 3, 4, 5, or 6,
n is 1, 2 or 3
s is 0, 1, 2, or 3,
t is 0, 1, 2, 3, or 4
p is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23;
and where B is an acidic group selected from

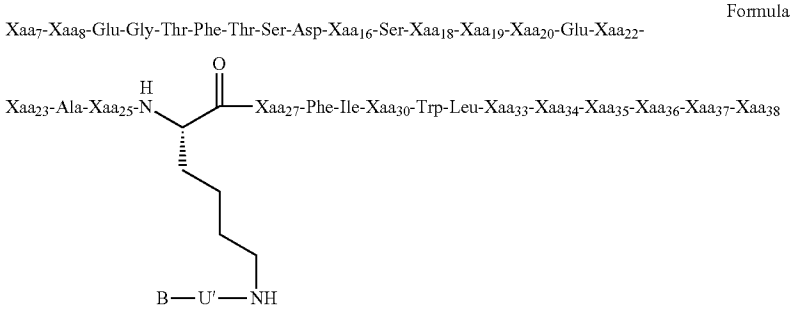


and

where l is 12, 13, 14, 15, 16, 17, 18, 19 or 20;

An embodiment provides a GLP-1 analog according to any of the above embodiments, which is a compound of formula I (SEQ ID No. 2):

Formula I



wherein

Xaa_7 is L-histidine, imidazopropionyl, α-hydroxy-histidine, D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, N^{α}-acetyl-histidine, N^{α}-formyl-histidine, α-fluoromethyl-histidine, α-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine

Xaa_8 is Ala, Gly, Val, Leu, Ile, Thr, Ser, Lys, Aib, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid;

Xaa_{16} is Val or Leu;

Xaa_{18} is Ser, Lys or Arg;

Xaa_{19} is Tyr or Gln;

Xaa_{20} is Leu or Met;

Xaa_{22} is Gly, Glu or Aib;

Xaa_{23} is Gln, Glu, Lys or Arg;

Xaa_{25} is Ala or Val;

Xaa_{27} is Glu or Leu;

Xaa_{30} is Ala, Glu or Arg;

Xaa_{33} is Val or Lys;

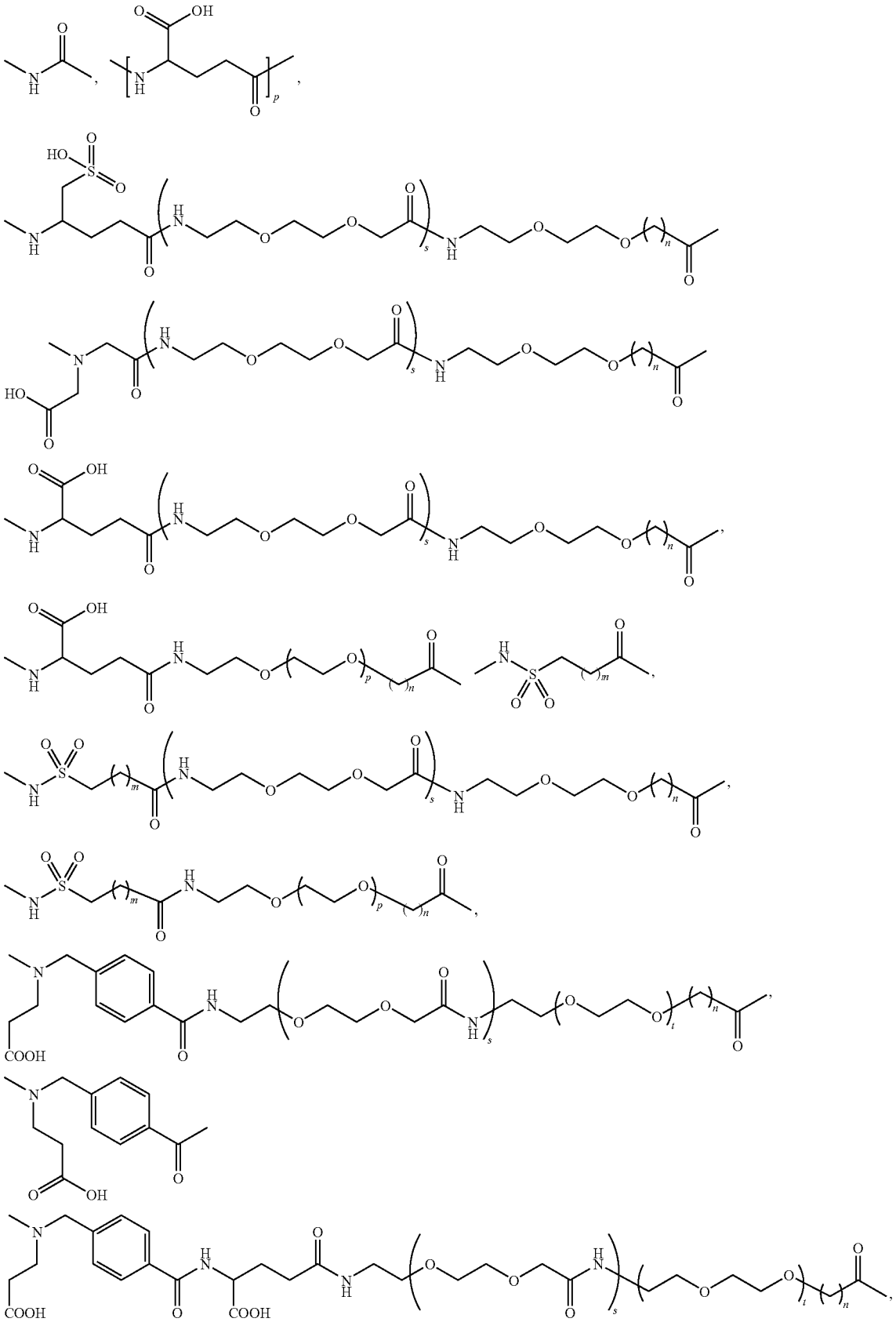
Xaa_{34} is Lys, Glu, Asn or Arg;

Xaa_{35} is Gly or Aib;

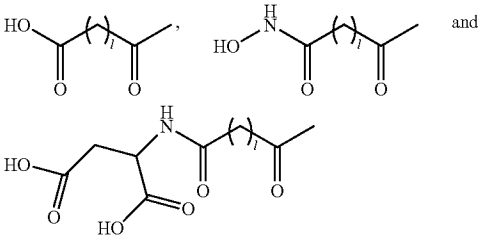
Xaa_{36} is Arg, Gly or Lys, or is absent;

Xaa_{37} is Gly, Ala, Glu, Pro, Lys, or is absent;

and B and U' together is the acylated moiety, where U' is selected from

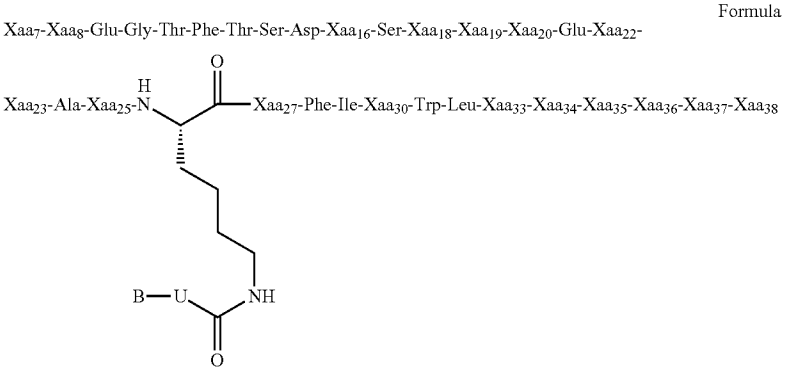


m is 0, 1, 2, 3, 4, 5, or 6,
n is 1, 2 or 3
s is 0, 1, 2, or 3,
t is 0, 1, 2, 3, or 4
p is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23;
and where B is an acidic group selected from



where l is 12, 13, 14, 15, 16, 17, 18, 19 or 20;

In an embodiment the invention provides a compound which is a compound of formula II (SEQ ID No. 3):



Formula II

Xaa$_7$-Xaa$_8$-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Xaa$_{16}$-Ser-Xaa$_{18}$-Xaa$_{19}$-Xaa$_{20}$-Glu-Xaa$_{22}$-Xaa$_{23}$-Ala-Xaa$_{25}$-N(H)-C(=O)-Xaa$_{27}$-Phe-Ile-Xaa$_{30}$-Trp-Leu-Xaa$_{33}$-Xaa$_{34}$-Xaa$_{35}$-Xaa$_{36}$-Xaa$_{37}$-Xaa$_{38}$

The formula II is identical to formula I as stated in an embodiment above, where the moiety B—U is replaced by B—U'. The difference being only the incorporation of the carboxy group in the U' relative to U, which is without the attaching carboxy group.

In formula II each of the Xaa's has the following meaning:

Xaa$_7$ is L-histidine, D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, N$^{\alpha}$-acetyl-histidine, α-fluoromethyl-histidine, α-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine;

Xaa$_8$ is Ala, Gly, Val, Leu, Ile, Lys, Aib, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid;

Xaa$_{16}$ is Val or Leu;

Xaa$_{18}$ is Ser, Lys or Arg;

Xaa$_{19}$ is Tyr or Gln;

Xaa$_{20}$ is Leu or Met;

Xaa$_{22}$ is Gly, Glu or Aib;

Xaa$_{23}$ is Gln, Glu, Lys or Arg;

Xaa$_{25}$ is Ala or Val;

Xaa$_{27}$ is Glu or Leu;

Xaa$_{30}$ is Ala, Glu or Arg;

Xaa$_{33}$ is Val or Lys;

Xaa$_{34}$ is Lys, Glu, Asn or Arg;

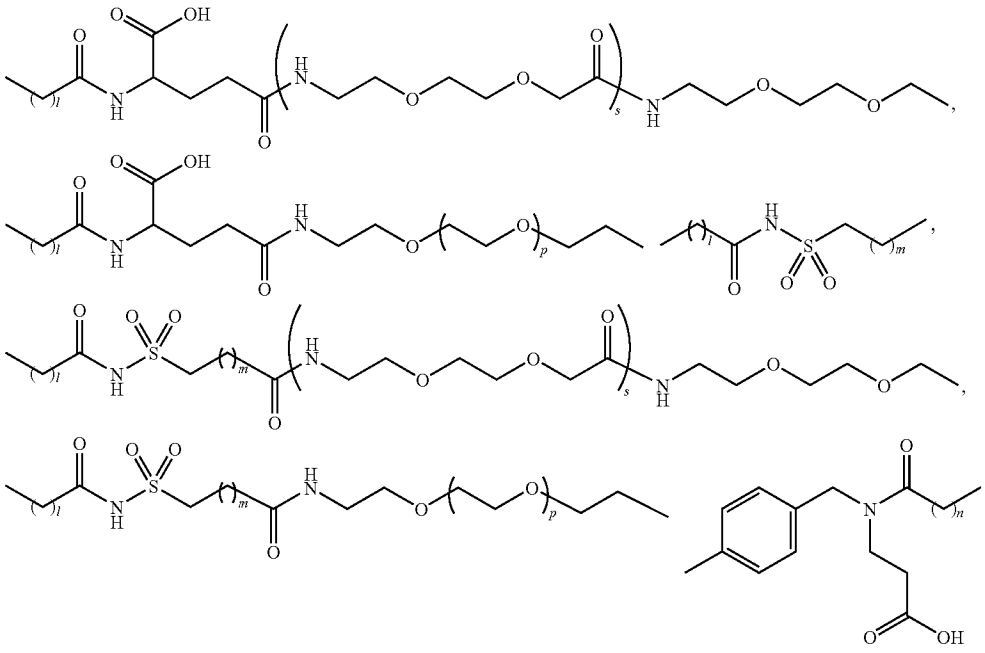
Xaa$_{35}$ is Gly or Aib;

Xaa$_{36}$ is Arg, Gly or Lys, or is absent;

Xaa$_{37}$ is Gly, Ala, Glu, Pro, Lys, or is absent;

Xaa$_{38}$ is Lys, Ser, amide or is absent;

and where U is a spacer selected from



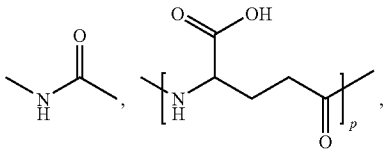
where n is 12, 13, 14, 15, 16, 17 or 18

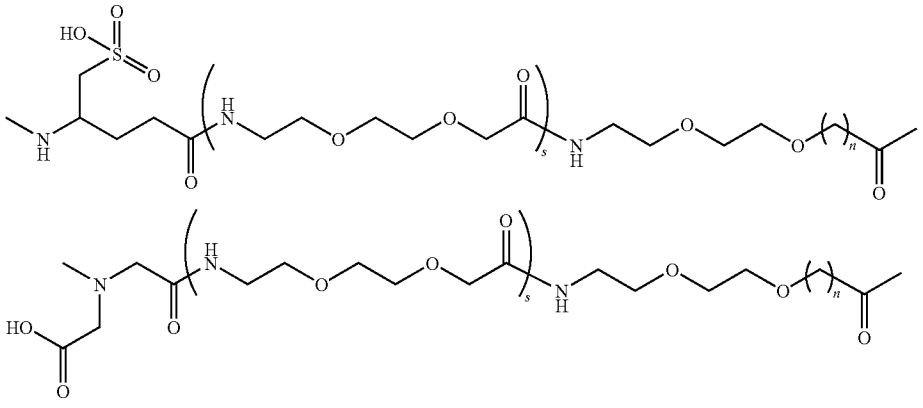
l is 12, 13, 14, 15, 16, 17 or 18,

m is 0, 1, 2, 3, 4, 5, or 6,

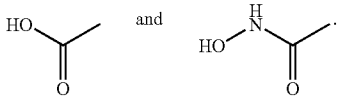
s is 0, 1, 2, or 3,

p is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23;





and where B is an acidic group selected from



In the embodiments below when referring to U' in formula I it is to be understood as also referring to formula II and U, with the only difference being the carboxy group.

An embodiment provides a GLP-1 analog according to the embodiments above, wherein U' is selected from

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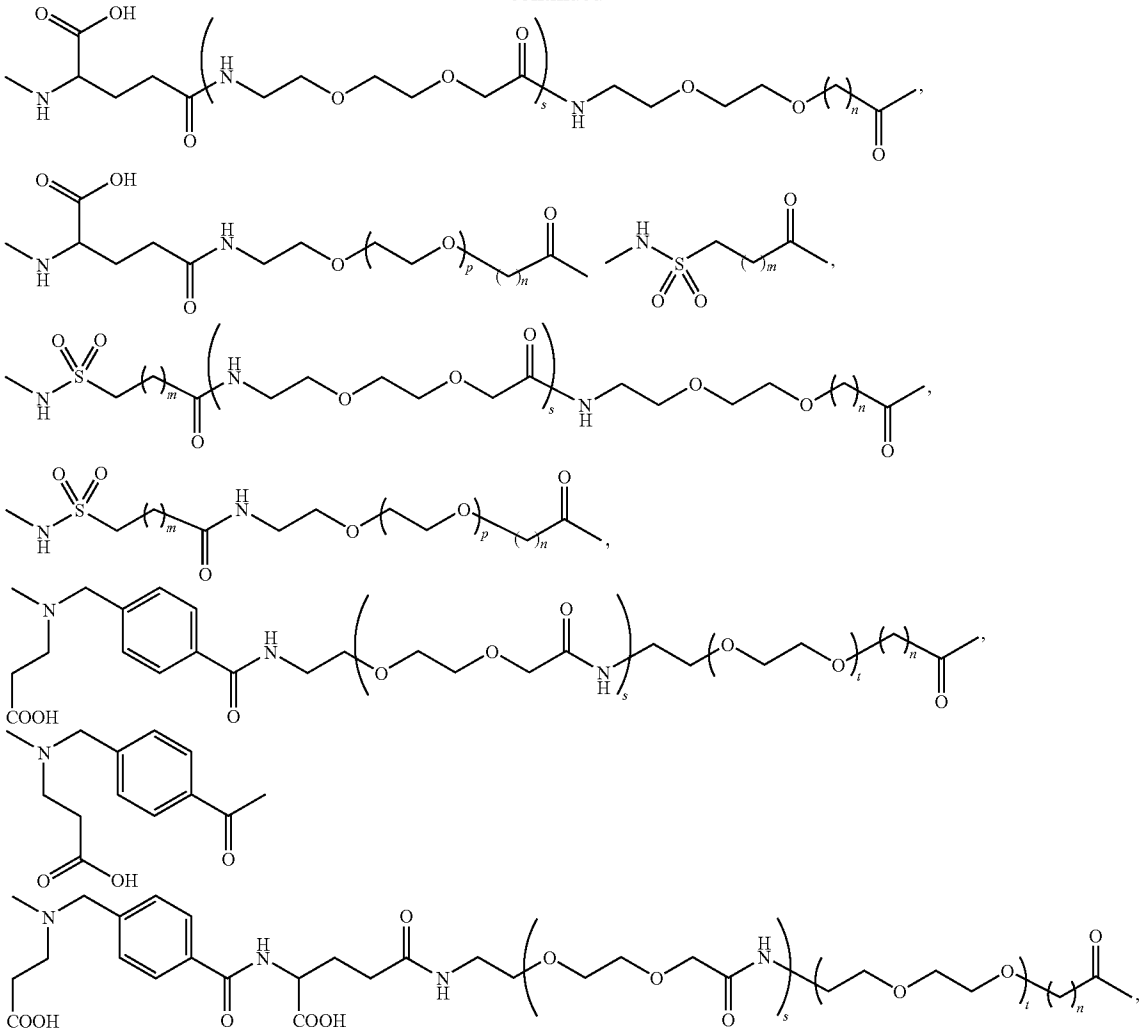


m is 2, 3, 4 or 5,

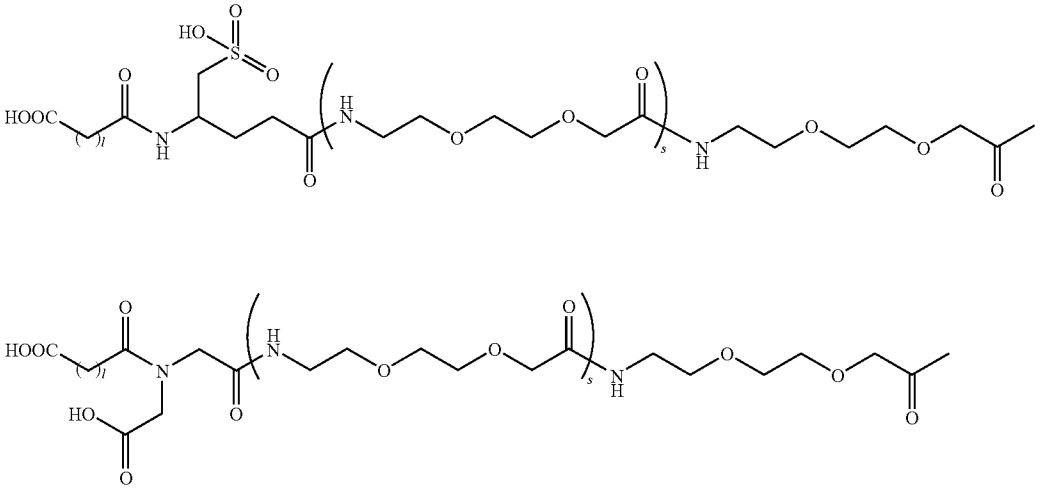
n is 1 or 2

s is 0, 1, or 2,

t is 0, 1, 2, or 3

p is 1, 2, 3, 4, 7, 11 or 23

An embodiment provides a GLP-1 analog according to the embodiments above, wherein B—U'— is



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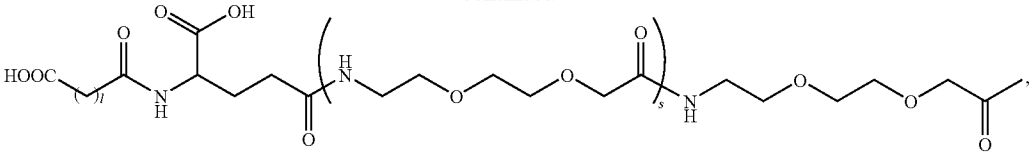
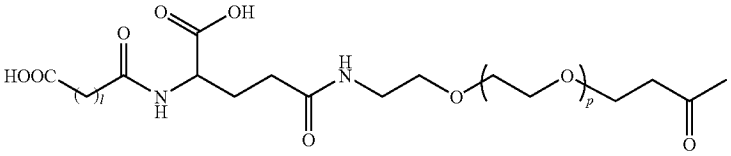
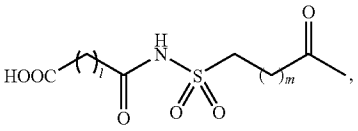
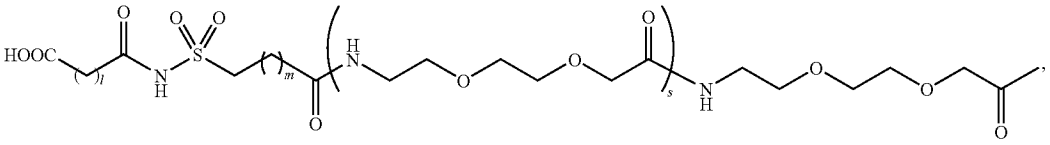
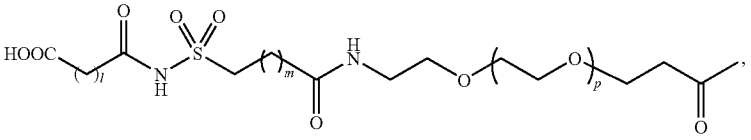
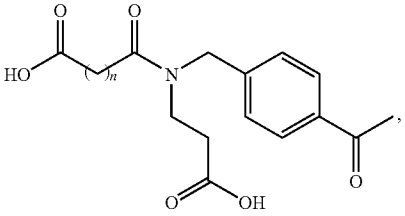


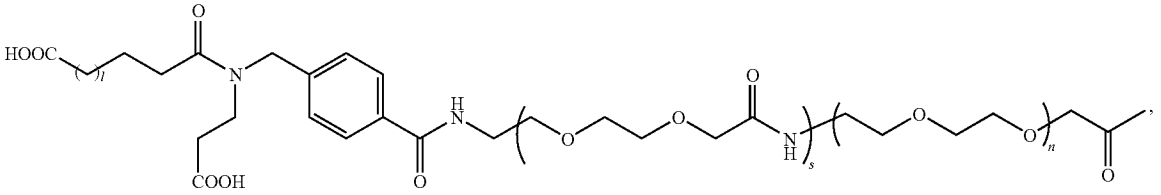


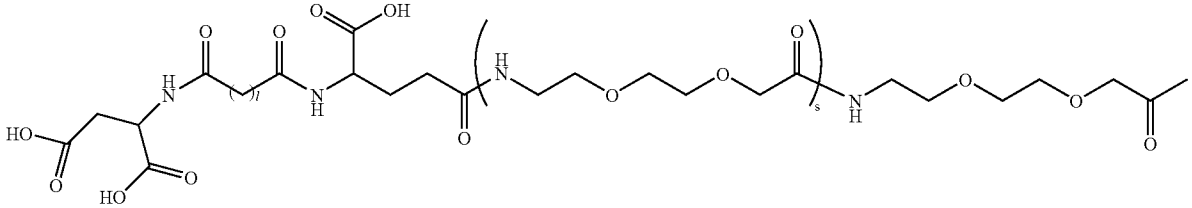


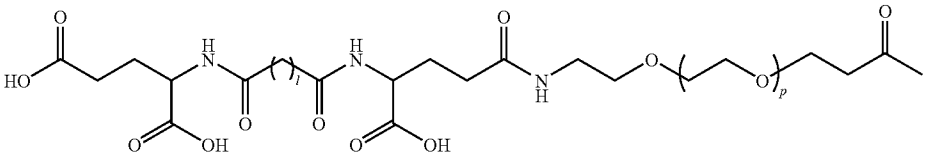












where l is 14, 15, 16, 17, 18, 19 or 20;

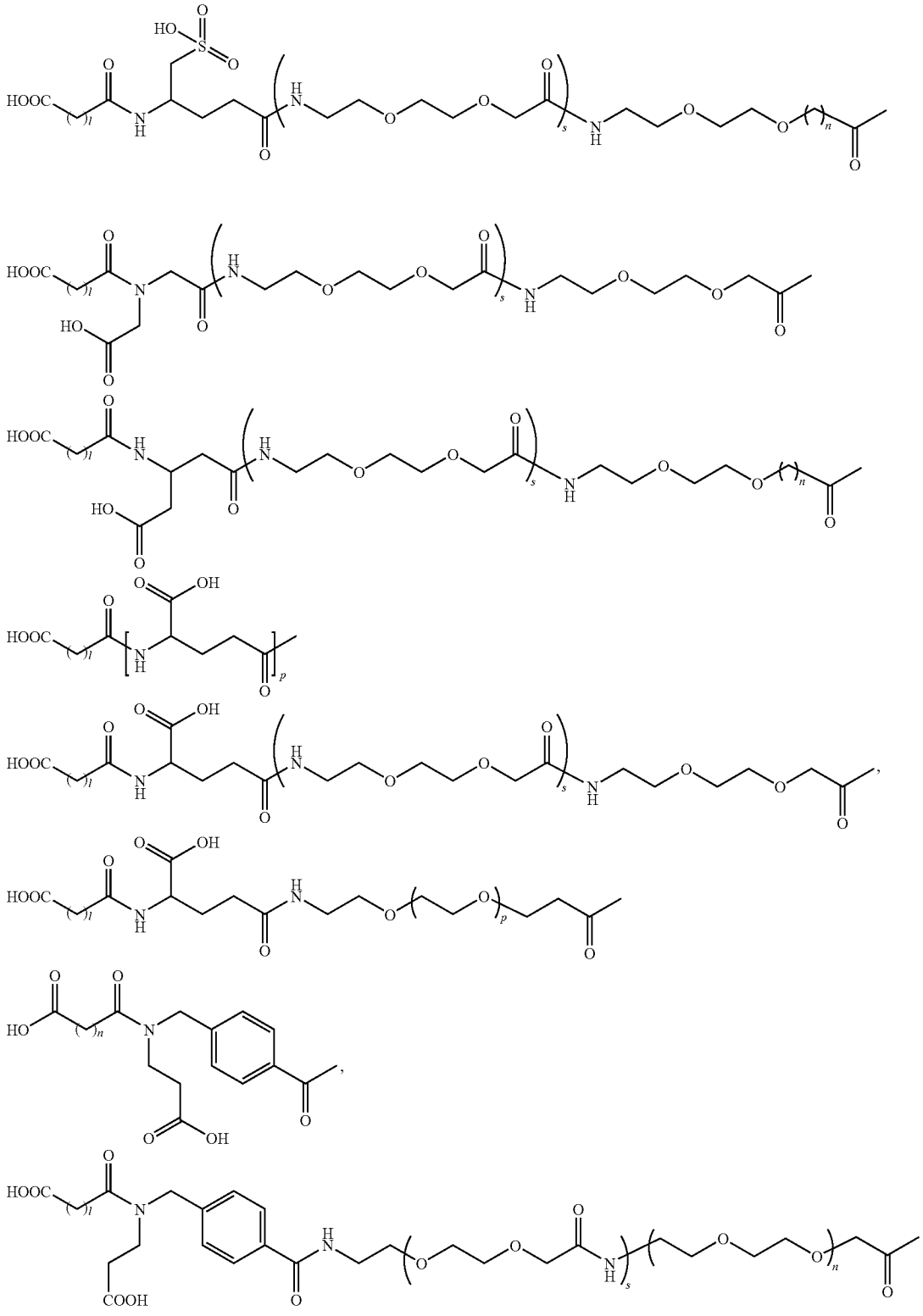
p is 1, 2, 3, 4, 7, 8, 9, 10, 11 or 12.

s is 0, 1 or 2

t is 0 or 1;

An embodiment according to the above wherein
where l is 14, 15, 16, 17 or 18
p is 1, 2, 3, 4 or 11;
s is 0, 1 or 2;
t is 0 or 1;

An embodiment provides a GLP-1 analog according to the embodiment above, wherein B—U' is



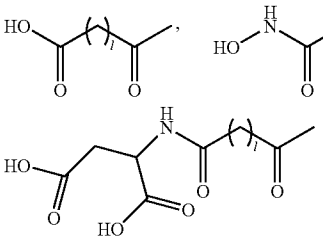
where l is 14, 15, 16, 17, 18, 19 or 20;

p is 1, 2, 3, or 4.

s is 0, 1 or 2

n is 0, 1 or 2

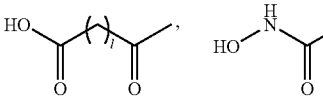
An embodiment according to any of the above embodiments is wherein B is



and

and l is 14, 16, 18 or 20;

An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein B is



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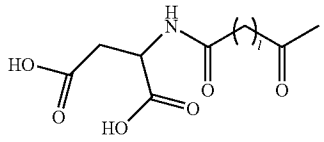


where l is 14, 15, or 16.

An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein s is 1.

An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein n is 1.

An embodiment provides a GLP-1 analog according any of the embodiments above, wherein l is 14, 15 or 16; In embodiments I is 17, 18, 19 or 20. In embodiments I is 15, 16 or 17. In embodiments I is 18, 19 or 20. In embodiments I is 14. In embodiments I is 16. In embodiments I is 18. In embodiments I is 20.

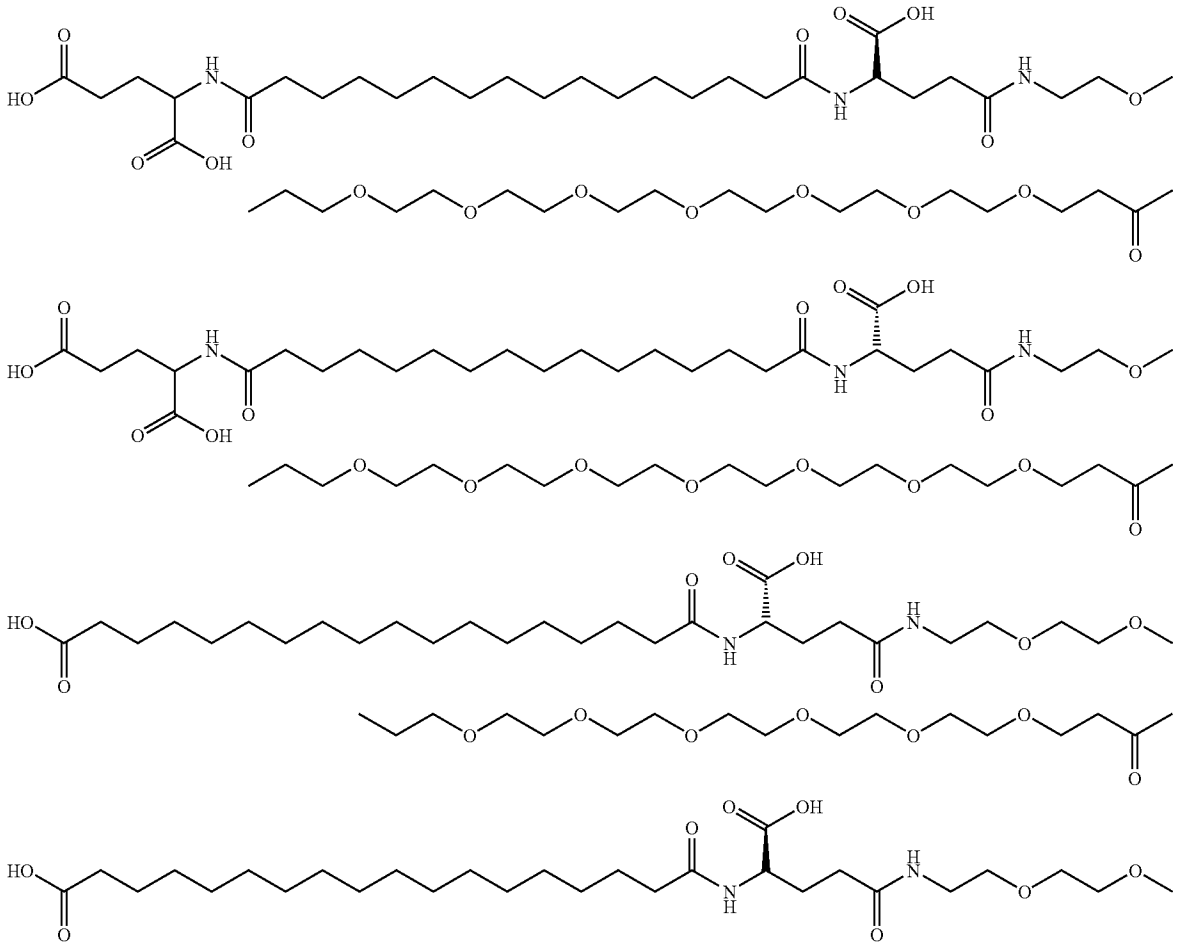
An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein p is 1.

An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein p is 2.

An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein p is 3.

An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein p is 4.

An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein B—U' is



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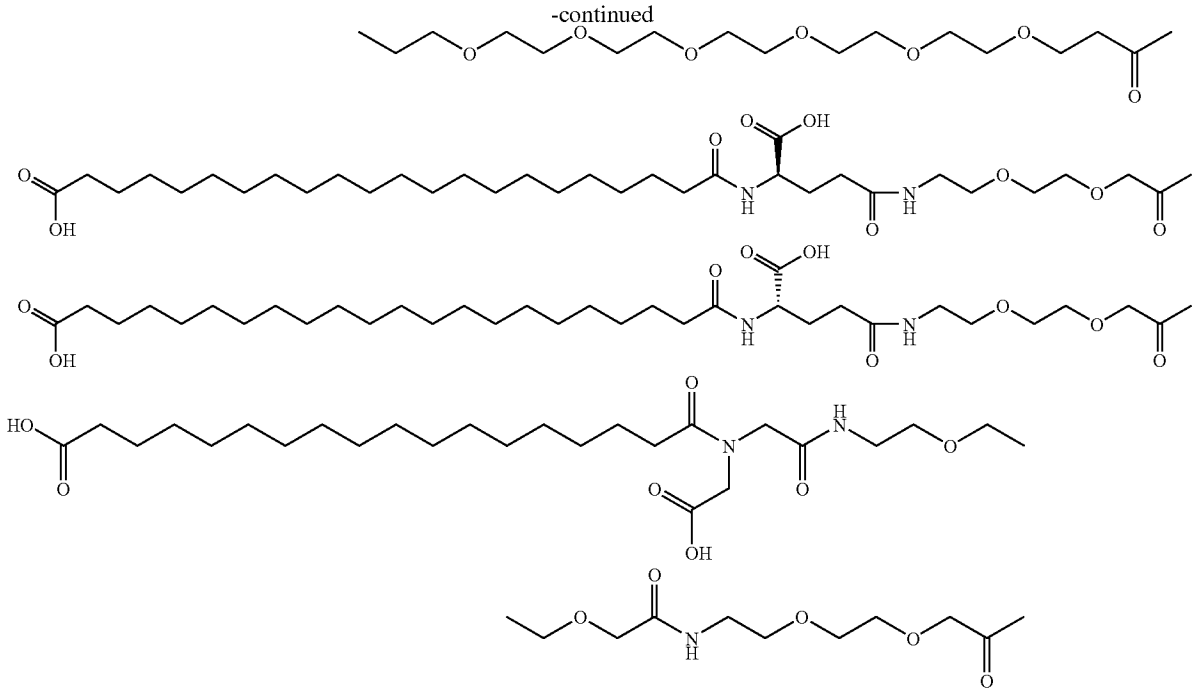
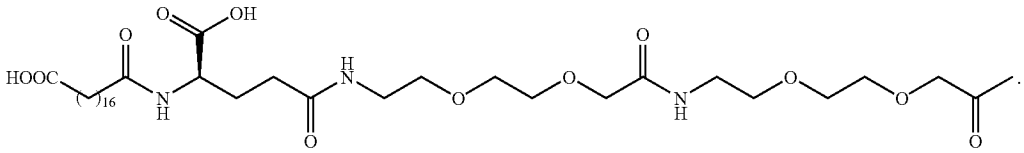
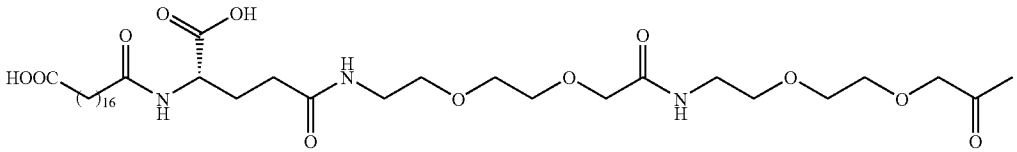


An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein B—U' is



An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein B—U' is



An embodiment provides a GLP-1 analog according to formula I above, wherein

Xaa_7 is His or desamino-histidine;

Xaa_8 is Ala, Gly, Val, Leu, Ile, Lys or Aib;

Xaa_{16} is Val;

Xaa_{18} is Ser;

Xaa_{19} is Tyr;

Xaa_{20} is Leu;

Xaa_{22} is Gly, Glu or Aib;

Xaa_{23} is Gln or Glu;

Xaa_{25} is Ala;

Xaa_{27} is Glu;

Xaa_{30} is Ala or Glu;

Xaa_{33} is Val;

Xaa_{34} is Lys or Arg;

Xaa_{35} is Gly or Aib;

Xaa_{36} is Arg or Lys

Xaa_{37} is Gly, amide or is absent;

An embodiment provides a GLP-1 analog according to formula I above, wherein

Xaa_7 is His

Xaa_8 is Gly, or Aib;

Xaa_{16} is Val;

Xaa_{18} is Ser;

Xaa_{19} is Tyr;

Xaa_{20} is Leu;

Xaa_{22} is Glu or Aib;

Xaa_{23} is Gln;

Xaa_{25} is Ala;

Xaa_{27} is Glu;

Xaa_{30} is Ala;
Xaa_{33} is Val;
Xaa_{34} is Lys or Arg;
Xaa_{35} is Gly or Aib;
Xaa_{36} is Arg
Xaa_{37} is Gly

An embodiment provides a GLP-1 analog according to any one of the above embodiments, wherein said GLP-1 analog comprises a modification of the N-terminal L-histidine in position 7 of the GLP-1(7-37) sequence.

An embodiment provides a GLP-1 analog according to the embodiment above, wherein said GLP-1 analog comprises imidazopropionyl^7, α-hydroxy-histidine^7 or N-methyl-histidine^7, D-histidine^7, desamino-histidine^7, 2-amino-histidine^7, β-hydroxy-histidine^7, homohistidine^7, N^{α}-acetyl-histidine^7, α-fluoromethyl-histidine^7, α-methyl-histidine^7, 3-pyridylalanine^7, 2-pyridylalanine^7 or 4-pyridylalanine^7.

An embodiment provides a GLP-1 analog according to any one of the embodiments above, wherein said GLP-1 analog comprises a substitution of the L-alanine in position 8 of the GLP-1(7-37) sequence for another amino acid residue.

An embodiment provides a GLP-1 analog according to the embodiment above, wherein said GLP-1 analog comprises Aib^8, Gly^8, Val^8, Ile^8, Leu^8, Ser^8, Thr^8, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid.

An embodiment provides a GLP-1 analog according to any of the embodiment above, wherein said GLP-1 analog comprises Aib^8;

In one embodiment of the invention said GLP-1 analog is Aib^8,Arg^{34}-GLP-1(7-37) or Aib^{8,22},Arg^{34}-GLP-1(7-37).

An embodiment provides a GLP-1 analog according to any of the above embodiments, wherein said GLP-1 analog comprises no more than fifteen amino acid residues which have been exchanged, added or deleted as compared to GLP-1(7-37) (SEQ ID No. 1),

An embodiment provides a GLP-1 analog according to the embodiment above, wherein no more than ten amino acid residues which have been exchanged, added or deleted as compared to GLP-1(7-37) (SEQ ID No. 1).

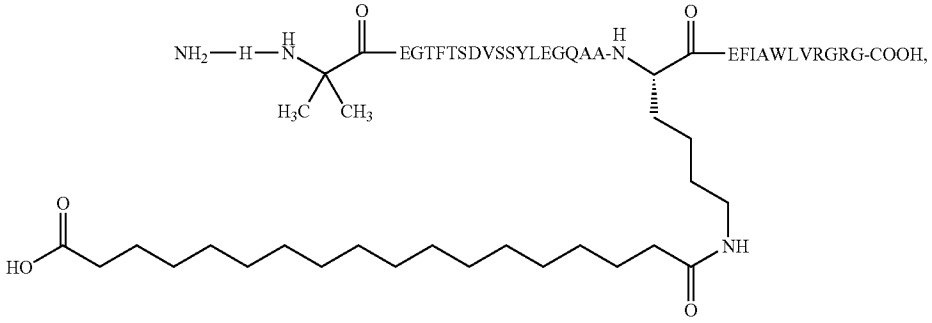
An embodiment provides a GLP-1 analog according to the embodiment above, wherein said GLP-1 analog comprises no more than six amino acid residues which have been exchanged, added or deleted as compared to GLP-1(7-37) (SEQ ID No. 1).

An embodiment provides a GLP-1 analog according to any of the above embodiments, wherein said GLP-1 analog comprises no more than 3 amino acid residues which are not encoded by the genetic code.

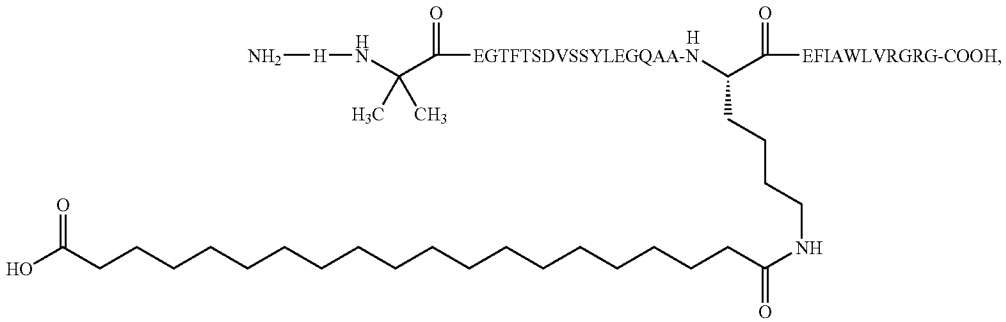
An embodiment provides a GLP-1 analog according to any of the above embodiments, wherein said GLP-1 analog comprises only one lysine residue.

An embodiment provides a GLP-1 analog according to any of the above embodiments, which is
Aib^8,Arg^{34}-GLP-1(7-37)
Aib^{8,22},Arg^{34}-GLP-1(7-37).
Arg^{34}-GLP-1(7-37).
[3-(4-Imidazolyl)Propionyl7,Arg34]GLP-1-(7-37)peptide
Gly^8,Arg^{34}-GLP-1(7-37)
Aib^8,Arg^{34}, Pro^{37}-GLP-1(7-37)
Aib^{8,22,27,30,35},Arg^{34},Pro^{37}-GLP-1(7-37)amide,
all of which are substituted by B—U' in position 26.

An embodiment provides a GLP-1 analog according to any one of the preceding embodiments, which is selected from



N-ε^{26}-(17-carboxyheptadecanoyl)-[Aib8,Arg34]GLP-1-(7-37)-peptide



N-ε^{26}-(19-carboxynonadecanoyl)-[Aib8,Arg34]GLP-1-(7-37)-peptide

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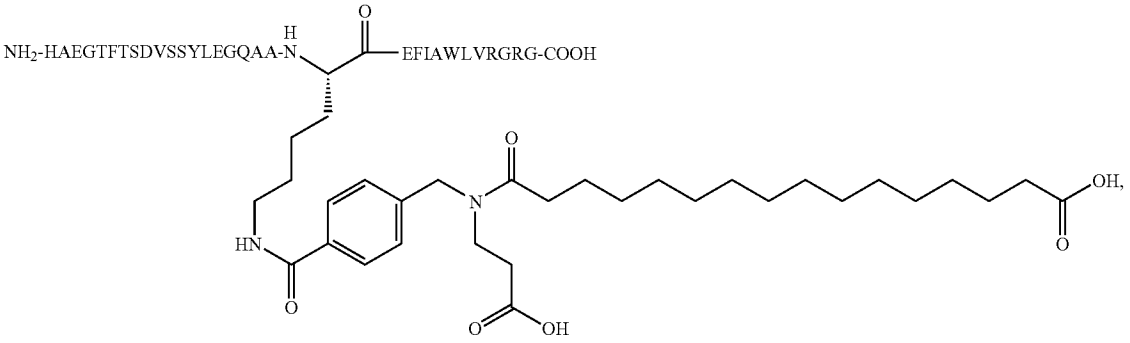
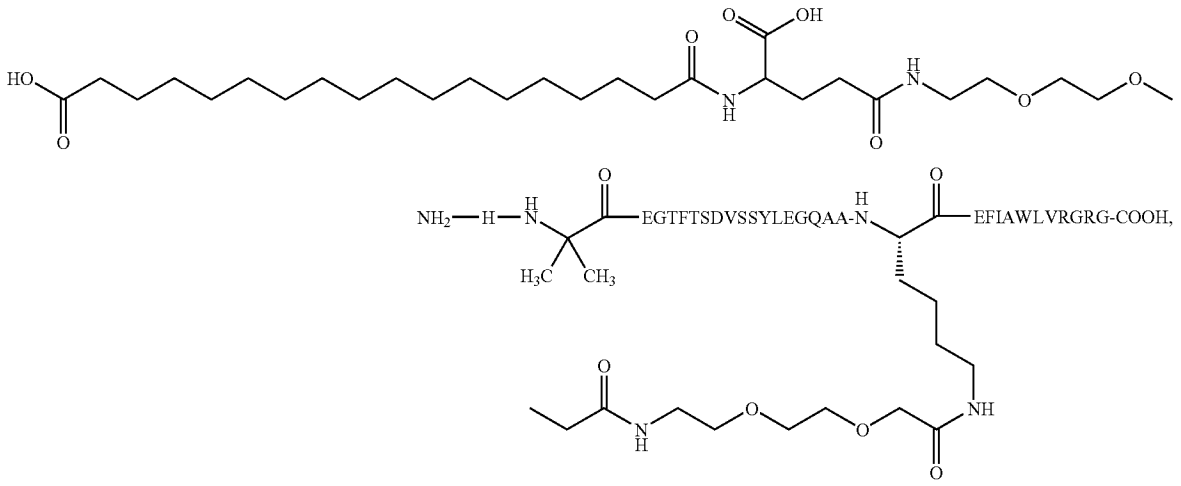
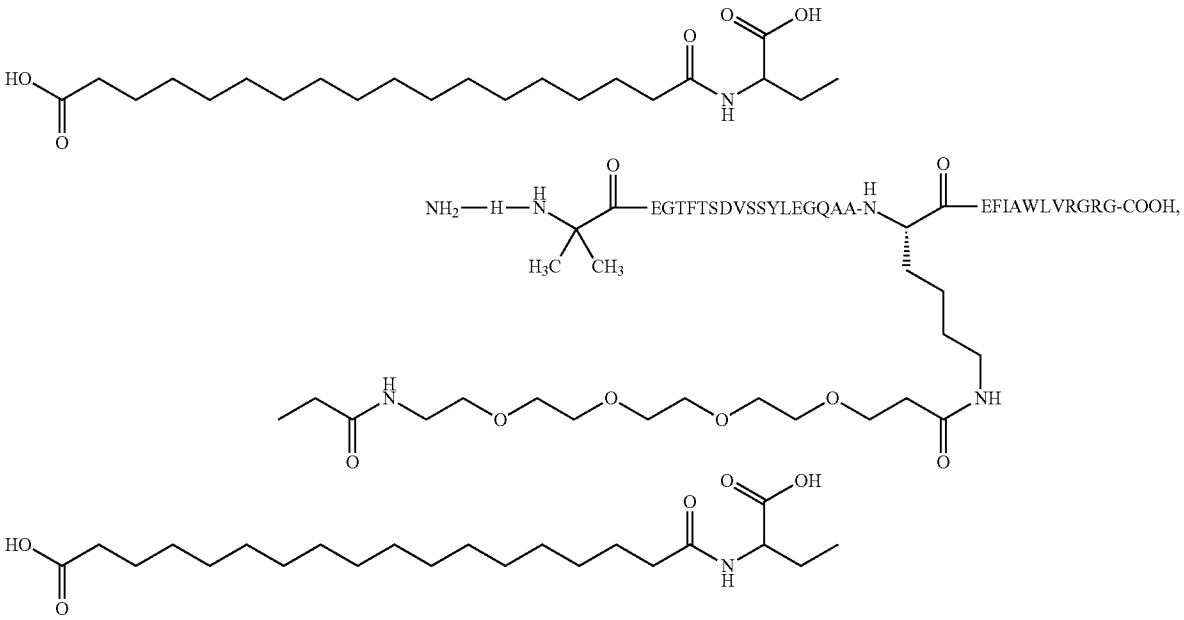


N-ε^{26}-(4-{[N-(carboxyethyl)-N-(15-carboxypentadecanoyl)amino]methyl}benzoyl)[Arg34]GLP-1-(7-37)



N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide



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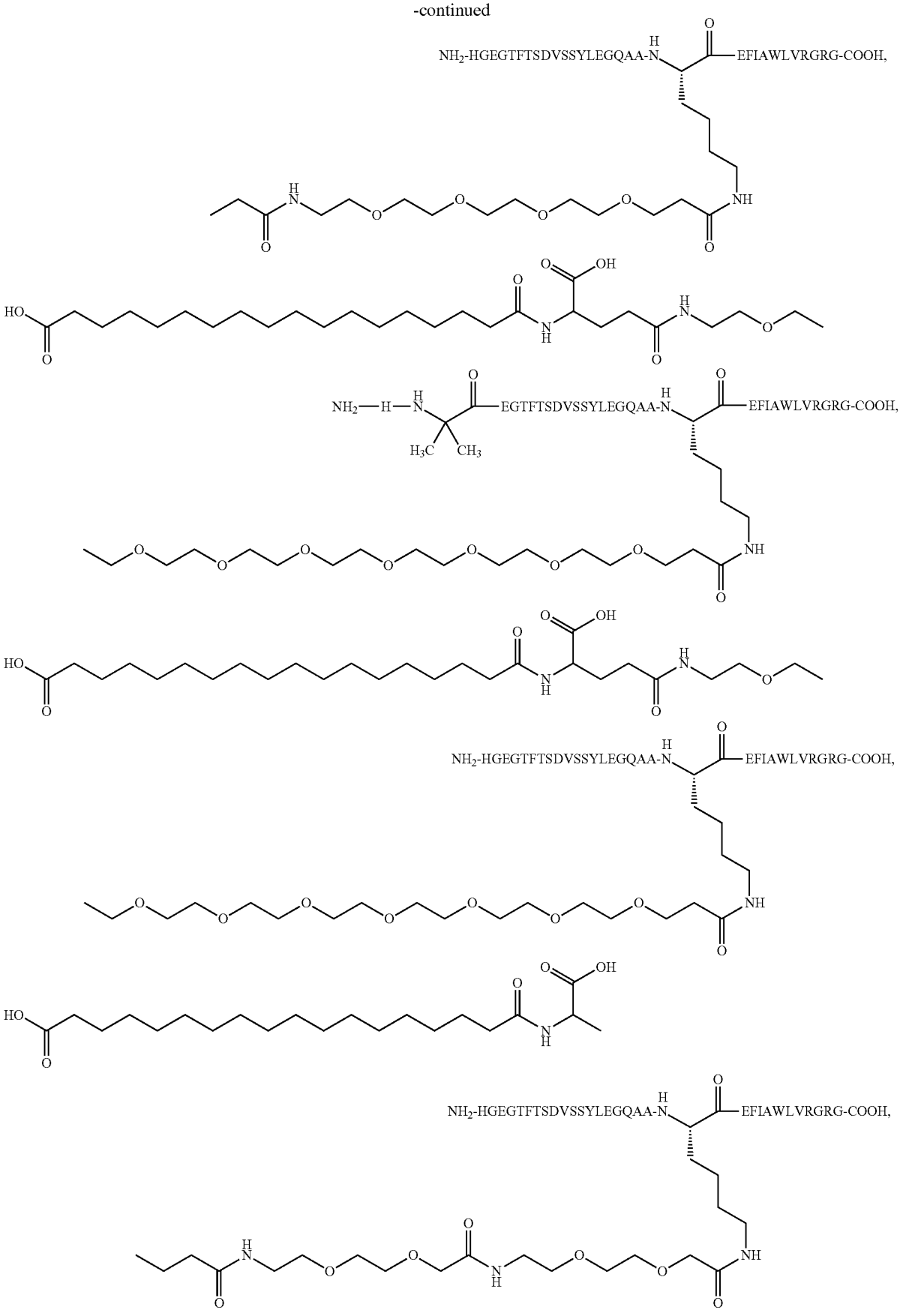
NH2-HGEGTFTSDVSSYLEGQAA-N
EFIAWLVRGRG-COOH,
HO
OH
NH2
H3C
CH3
NH

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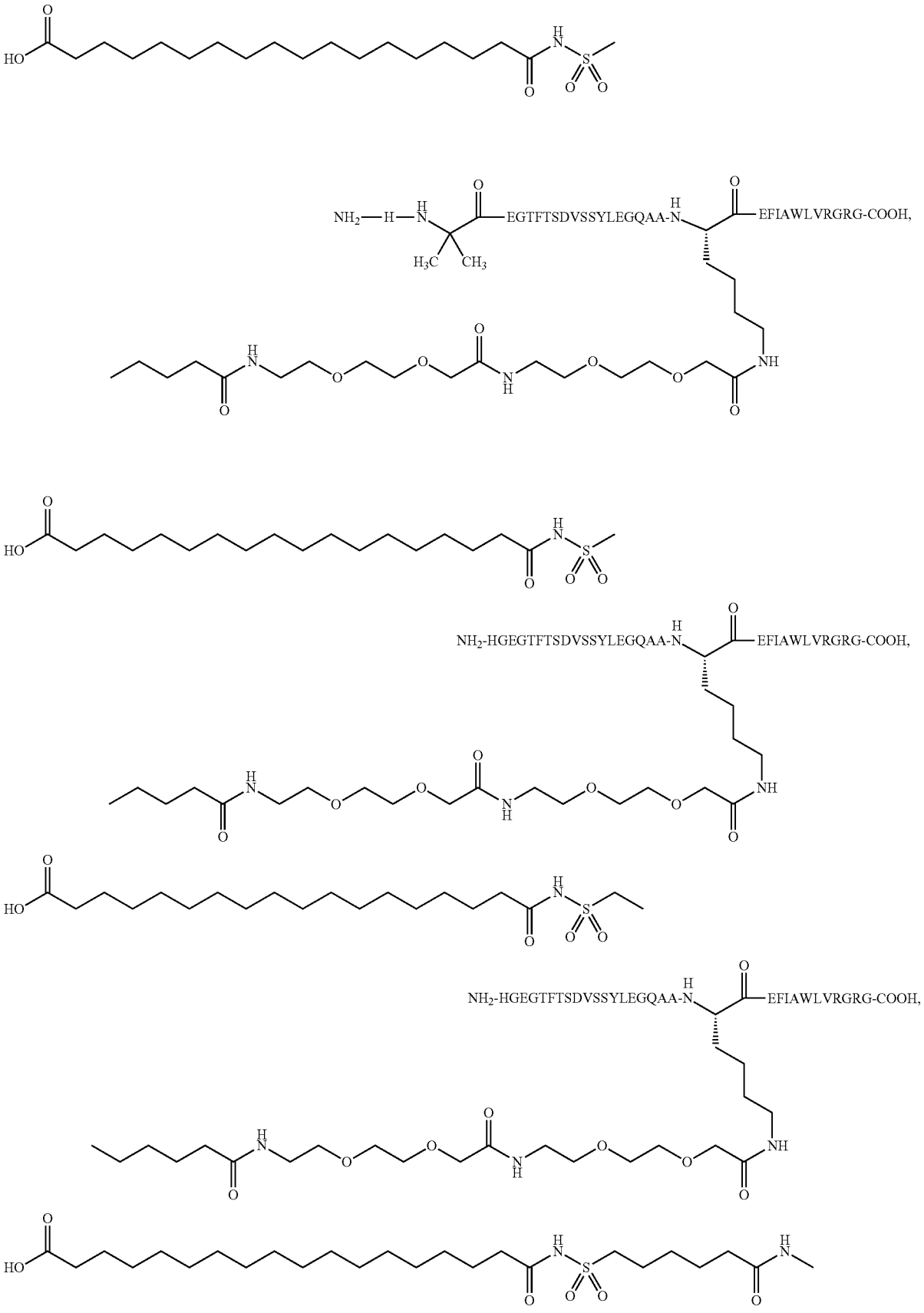
NH2—H—
EGTFTSDVSSYLEGQAA-
EFIAWLVRGRG-COOH,
H3C
CH3
HO
NH2-HGEGTFTSDVSSYLEGQAA-
EFIAWLVRGRG-COOH,
HO
NH2-HGEGTFTSDVSSYLEGQAA-
EFIAWLVRGRG-COOH,
HO

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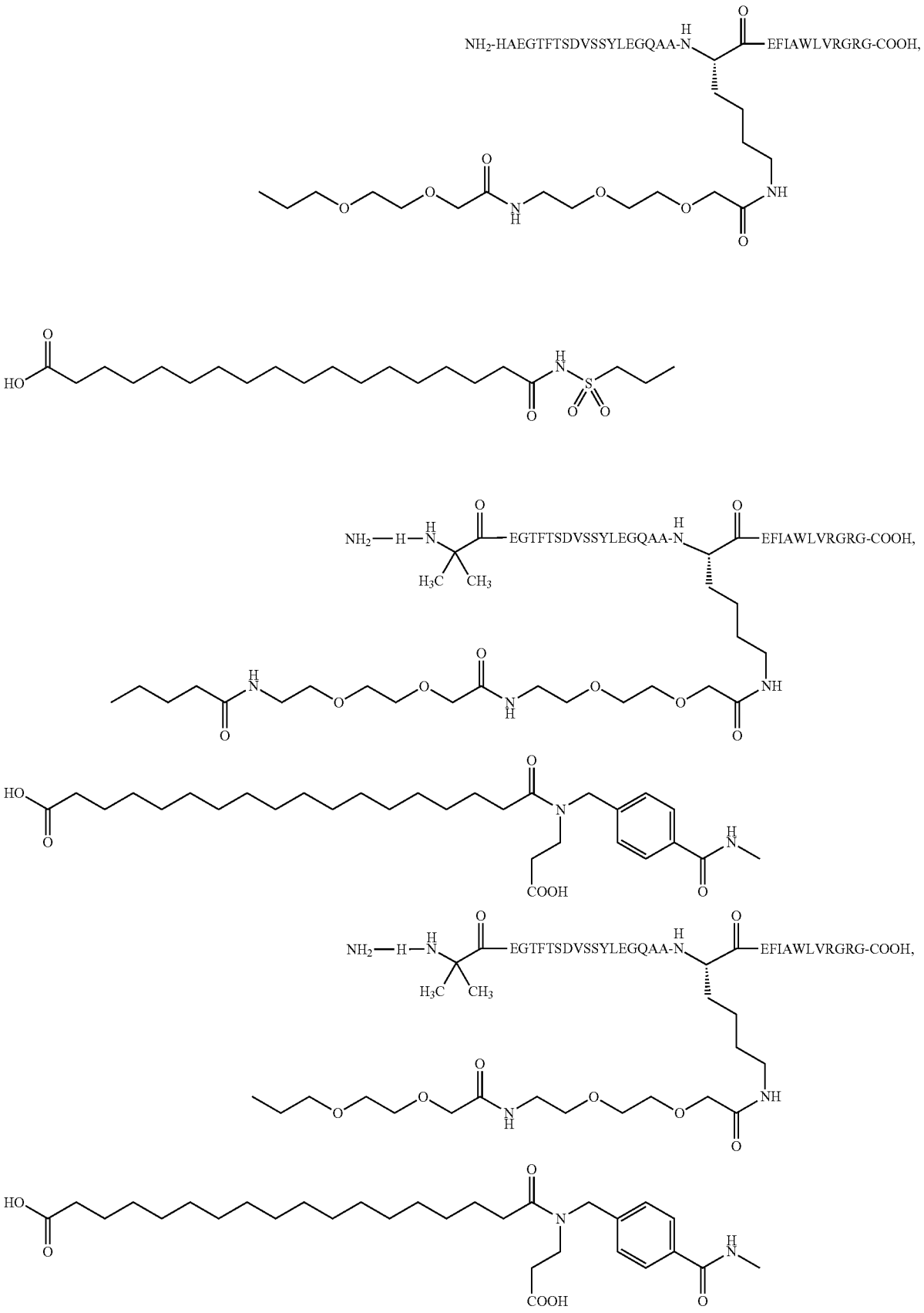
NH2-HAEGTFTSDVSSYLEGQAA-N
EFIAWLVRGRG-COOH,
HO
NH2—H—N
EGTFTSDVSSYLEGQAA-N
EFIAWLVRGRG-COOH,
H3C
CH3
COOH
NH2—H—N
EGTFTSDVSSYLEGQAA-N
EFIAWLVRGRG-COOH,
H3C
CH3
COOH

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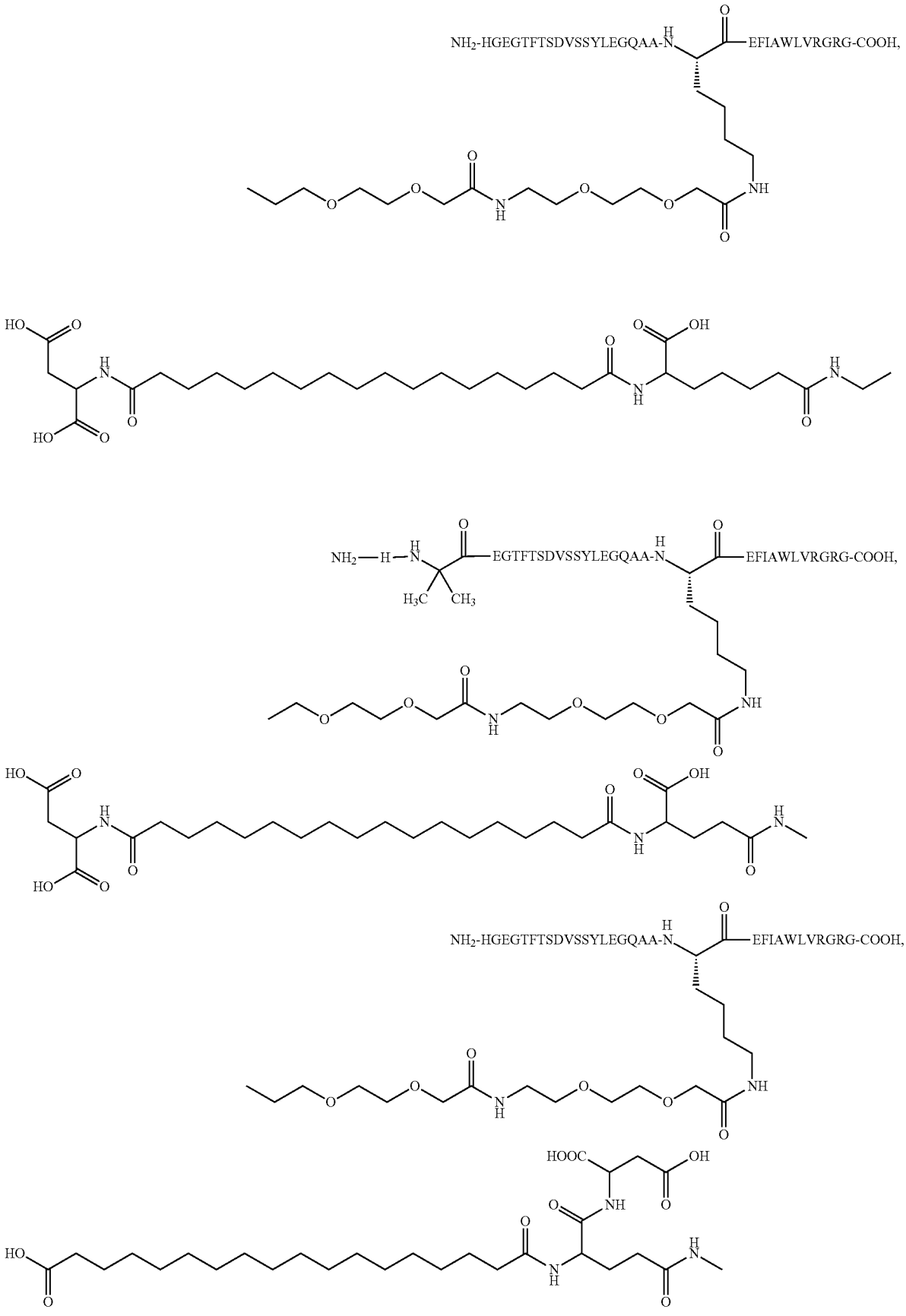
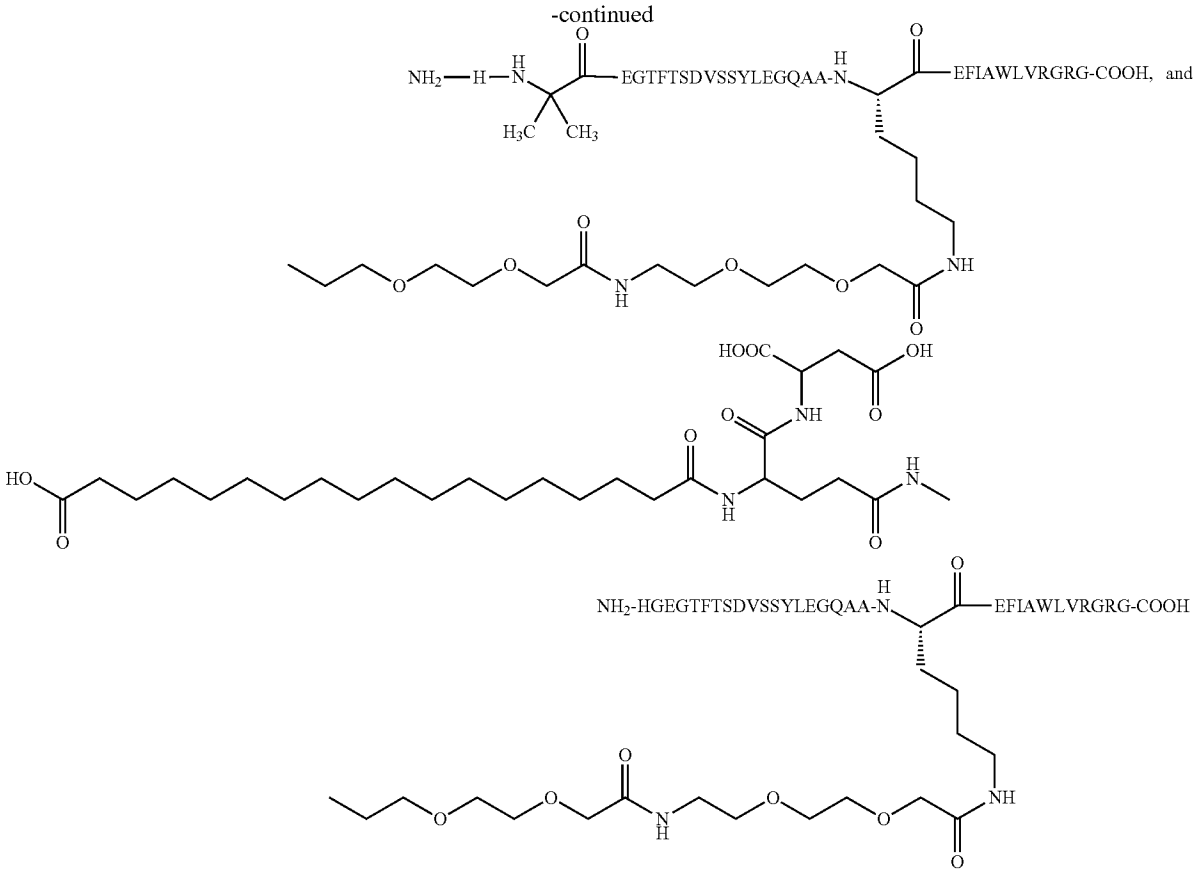

NH2-HGEGTFTSDVSSYLEGQAA-N
EFIAWLVRGRG-COOH,
NH2—H—N
EGTFTSDVSSYLEGQAA-N
EFIAWLVRGRG-COOH,
H3C
CH3
NH2-HGEGTFTSDVSSYLEGQAA-N
EFIAWLVRGRG-COOH,
HOOC
OH
NH
HO
OH
NH
O

-continued



An embodiment provides a method for increasing the time of action in a patient of a GLP-1 analog, characterised in acylating said GLP-1 analog with a moiety B—U as disclosed in any of the preceding embodiments, on the lysine residue in position 26 of said GLP-1 analog.

An embodiment provides a method for increasing the time of action in a patient of a GLP-1 analog to more than about 40 hours, characterised in modifying at least one of the amino acid residues in positions 7 and 8 of a GLP-1(7-37) peptide or an analog thereof, and acylating said GLP-1 analog with a moiety B—U'— as disclosed in any of the preceding embodiments on the lysine residue in position 26 of said GLP-1 analog.

An embodiment provides a pharmaceutical composition comprising a compound according to any one the embodiments above, and a pharmaceutically acceptable excipient.

An embodiment provides a pharmaceutical composition according to the embodiment above, which is suited for parenteral administration.

An embodiment provides the use of a compound according to any one of the embodiments above for the preparation of a medicament.

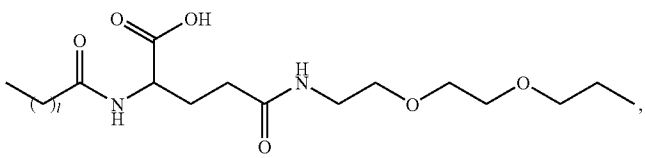
An embodiment provides the use of a compound according to any one of the embodiments above for the preparation of a medicament for the treatment or prevention of hyperglycemia, type 2 diabetes, impaired glucose tolerance, type 1 diabetes, obesity, hypertension, syndrome X, dyslipidemia, cognitive disorders, atheroschlerosis, myocardial infarction, coronary heart disease and other cardiovascular disorders, stroke, inflammatory bowel syndrome, dyspepsia and gastric ulcers.

An embodiment provides the use of a compound according to any one of the embodiments above for the preparation of a medicament for delaying or preventing disease progression in type 2 diabetes.

An embodiment provides the use of a compound according to any one of the embodiments above for the preparation of a medicament for decreasing food intake, decreasing β-cell apoptosis, increasing β-cell function and β-cell mass, and/or for restoring glucose sensitivity to β-cells.

In an embodiment the invention provides a compound according to the embodiments above, wherein said GLP-1 analog is Aib^{8},Arg^{34}-GLP-1(7-37) or $Aib^{8,22}$,Arg^{34}-GLP-1 (7-37) attached to a linker B—U';

In an embodiment of Formula II, B—U represents



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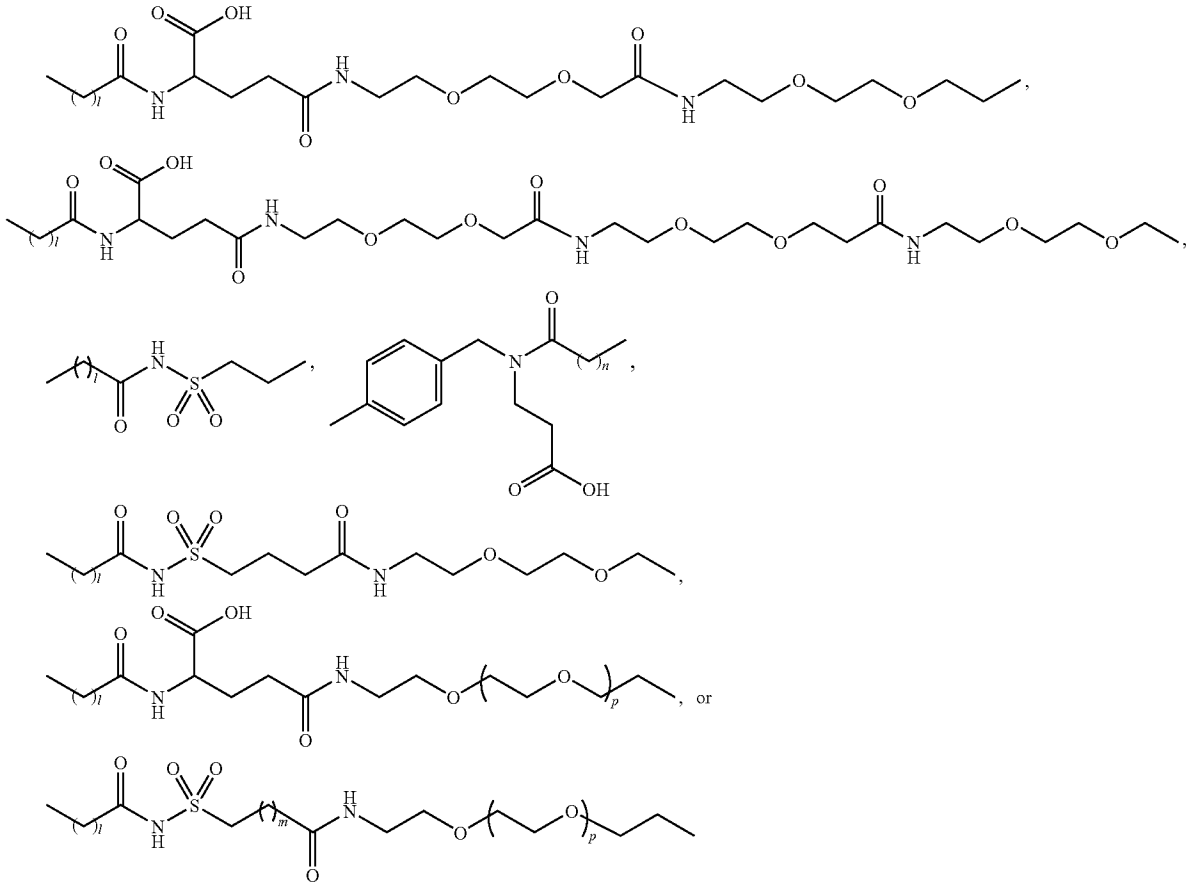


where l is 14, 15 or 16;

n is 15, 16, 17 or 18;

p is 3, 7, 11 or 24.

In embodiments the invention provides a compound according to any one of the embodiments above, wherein said diacid comprises a dicarboxylic acid.

In embodiments the invention provides a compound according to any one of the embodiments above, wherein the acylation group comprises a straight-chain or branched alkane α,ω-dicarboxylic acid.

In embodiments the invention provides compound according to the embodiment above,

wherein the acylation group comprises the structure HOOC—$(CH_2)_n$CO—, wherein n is 12 to 20.

In embodiments the invention provides a compound according to the embodiment above, wherein the acylation group comprises a structure selected from HOOC—$(CH_2)_{14}$CO—, HOOC—$(CH_2)_{15}$CO—, HOOC—$(CH_2)_{16}$CO—, HOOC—$(CH_2)_{17}$CO—, and HOOC—$(CH_2)_{18}$CO—.

In embodiments the invention provides a compound according to the embodiment above, wherein the acylation group comprises the structure HOOC—$(CH_2)_{16}$CO—.

Another object of the present invention is to provide a pharmaceutical formulation comprising a compound according to the present invention which is present in a concentration from 0.1 mg/ml to 25 mg/ml, and wherein said formulation has a pH from 3.0 to 9.0. The formulation may further comprise a buffer system, preservative(s), tonicity agent(s), chelating agent(s), stabilizers and surfactants. In one embodiment of the invention the pharmaceutical formulation is an aqueous formulation, i.e. formulation comprising water. Such formulation is typically a solution or a suspension. In a further embodiment of the invention the pharmaceutical formulation is an aqueous solution. The term "aqueous formulation" is defined as a formulation comprising at least 50% w/w water. Likewise, the term "aqueous solution" is defined as a solution comprising at least 50% w/w water, and the term "aqueous suspension" is defined as a suspension comprising at least 50% w/w water.

In another embodiment the pharmaceutical formulation is a freeze-dried formulation, whereto the physician or the patient adds solvents and/or diluents prior to use.

In another embodiment the pharmaceutical formulation is a dried formulation (e.g. freeze-dried or spray-dried) ready for use without any prior dissolution.

In a further aspect the invention relates to a pharmaceutical formulation comprising an aqueous solution of a compound according to the present invention, and a buffer, wherein said compound is present in a concentration from 0.1 mg/ml or above, and wherein said formulation has a pH from about 3.0 to about 9.0.

In another embodiment of the invention the pH of the formulation is from about 7.0 to about 9.5. In another embodiment of the invention the pH of the formulation is from about 3.0 to about 7.0. In another embodiment of the invention the pH of the formulation is from about 5.0 to about 7.5. In another embodiment of the invention the pH of the formulation is from about 7.5 to about 9.0. In another embodiment of the invention the pH of the formulation is from about 7.5 to about 8.5. In another embodiment of the invention the pH of

the formulation is from about 6.0 to about 7.5. In another embodiment of the invention the pH of the formulation is from about 6.0 to about 7.0. In another embodiment the pharmaceutical formulation is from 8.0 to 8.5.

In a further embodiment of the invention the buffer is selected from the group consisting of sodium acetate, sodium carbonate, citrate, glycylglycine, histidine, glycine, lysine, arginine, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium phosphate, and tris(hydroxymethyl)-aminomethan, bicine, tricine, malic acid, succinate, maleic acid, fumaric acid, tartaric acid, aspartic acid or mixtures thereof. Each one of these specific buffers constitutes an alternative embodiment of the invention.

In a further embodiment of the invention the formulation further comprises a pharmaceutically acceptable preservative. In a further embodiment of the invention the preservative is selected from the group consisting of phenol, o-cresol, m-cresol, p-cresol, methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, 2-phenoxyethanol, butyl p-hydroxybenzoate, 2-phenylethanol, benzyl alcohol, chlorobutanol, and thiomerosal, bronopol, benzoic acid, imidurea, chlorohexidine, sodium dehydroacetate, chlorocresol, ethyl p-hydroxybenzoate, benzethonium chloride, chlorphenesine (3p-chlorphenoxypropane-1,2-diol) or mixtures thereof. In an embodiment the preservative is phenol or m-cresol. In a further embodiment of the invention the preservative is present in a concentration from 0.1 mg/ml to 20 mg/ml. In a further embodiment of the invention the preservative is present in a concentration from 0.1 mg/ml to 5 mg/ml. In a further embodiment of the invention the preservative is present in a concentration from 5 mg/ml to 10 mg/ml. In a further embodiment of the invention the preservative is present in a concentration from 10 mg/ml to 20 mg/ml. Each one of these specific preservatives constitutes an alternative embodiment of the invention. The use of a preservative in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: *The Science and Practice of Pharmacy,* 19th edition, 1995.

In a further embodiment of the invention the formulation further comprises an isotonic agent. In a further embodiment of the invention the isotonic agent is selected from the group consisting of a salt (e.g. sodium chloride), a sugar or sugar alcohol, an amino acid (e.g. L-glycine, L-histidine, arginine, lysine, isoleucine, aspartic acid, tryptophan, threonine), an alditol (e.g. glycerol (glycerine), 1,2-propanediol (propyleneglycol), 1,3-propanediol, 1,3-butanediol) polyethyleneglycol (e.g. PEG400), or mixtures thereof. In an embodiment the isotoncity agent is propyleneglycol. Any sugar such as mono-, di-, or polysaccharides, or water-soluble glucans, including for example fructose, glucose, mannose, sorbose, xylose, maltose, lactose, sucrose, trehalose, dextran, pullulan, dextrin, cyclodextrin, soluble starch, hydroxyethyl starch and carboxymethylcellulose-Na may be used. In one embodiment the sugar additive is sucrose. Sugar alcohol is defined as a C4-C8 hydrocarbon having at least one —OH group and includes, for example, mannitol, sorbitol, inositol, galactitol, dulcitol, xylitol, and arabitol. In one embodiment the sugar alcohol additive is mannitol. The sugars or sugar alcohols mentioned above may be used individually or in combination. There is no fixed limit to the amount used, as long as the sugar or sugar alcohol is soluble in the liquid preparation and does not adversely effect the stabilizing effects achieved using the methods of the invention. In one embodiment, the sugar or sugar alcohol concentration is between about 1 mg/ml and about 150 mg/ml. In a further embodiment of the invention the isotonic agent is present in a concentration from 1 mg/ml to 50 mg/ml. In a further embodiment of the invention the isotonic agent is present in a concentration from 1 mg/ml to 7 mg/ml. In an embodiment of the invention the isotonic agent is present in a concentration from 5 mg/ml to 7 mg/ml. In a further embodiment of the invention the isotonic agent is present in a concentration from 8 mg/ml to 24 mg/ml. In a further embodiment of the invention the isotonic agent is present in a concentration from 25 mg/ml to 50 mg/ml. Each one of these specific isotonic agents constitutes an alternative embodiment of the invention. The use of an isotonic agent in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: *The Science and Practice of Pharmacy,* 19th edition, 1995.

In a further embodiment of the invention the formulation further comprises a chelating agent. In a further embodiment of the invention the chelating agent is selected from salts of ethylenediaminetetraacetic acid (EDTA), citric acid, and aspartic acid, and mixtures thereof. In a further embodiment of the invention the chelating agent is present in a concentration from 0.1 mg/ml to 5 mg/ml. In a further embodiment of the invention the chelating agent is present in a concentration from 0.1 mg/ml to 2 mg/ml. In a further embodiment of the invention the chelating agent is present in a concentration from 2 mg/ml to 5 mg/ml. Each one of these specific chelating agents constitutes an alternative embodiment of the invention. The use of a chelating agent in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: *The Science and Practice of Pharmacy,* 19th edition, 1995.

In a further embodiment of the invention the formulation further comprises a stabilizer. The use of a stabilizer in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: *The Science and Practice of Pharmacy,* 19th edition, 1995.

More particularly, compositions of the invention are stabilized liquid pharmaceutical compositions whose therapeutically active components include a polypeptide that possibly exhibits aggregate formation during storage in liquid pharmaceutical formulations. By "aggregate formation" is intended a physical interaction between the polypeptide molecules that results in formation of oligomers, which may remain soluble, or large visible aggregates that precipitate from the solution. By "during storage" is intended a liquid pharmaceutical composition or formulation once prepared, is not immediately administered to a subject. Rather, following preparation, it is packaged for storage, either in a liquid form, in a frozen state, or in a dried form for later reconstitution into a liquid form or other form suitable for administration to a subject. By "dried form" is intended the liquid pharmaceutical composition or formulation is dried either by freeze drying (i.e., lyophilization; see, for example, Williams and Polli (1984) J. Parenteral Sci. Technol. 38:48-59), spray drying (see Masters (1991) in Spray-Drying Handbook (5th ed; Longman Scientific and Technical, Essez, U.K.), pp. 491-676; Broadhead et al. (1992) Drug Devel. Ind. Pharm. 18:1169-1206; and Mumenthaler et al. (1994) Pharm. Res. 11:12-20), or air drying (Carpenter and Crowe (1988) Cryobiology 25:459-470; and Roser (1991) Biopharm. 4:47-53). Aggregate formation by a polypeptide during storage of a liquid pharmaceutical composition can adversely affect biological activity of that polypeptide, resulting in loss of therapeutic efficacy of the pharmaceutical composition. Furthermore, aggregate formation may cause other problems such as blockage of tubing, membranes, or pumps when the polypeptide-containing pharmaceutical composition is administered using an infusion system.

The pharmaceutical compositions of the invention may further comprise an amount of an amino acid base sufficient

to decrease aggregate formation by the polypeptide during storage of the composition. By "amino acid base" is intended an amino acid or a combination of amino acids, where any given amino acid is present either in its free base form or in its salt form. Where a combination of amino acids is used, all of the amino acids may be present in their free base forms, all may be present in their salt forms, or some may be present in their free base forms while others are present in their salt forms. In one embodiment, amino acids to use in preparing the compositions of the invention are those carrying a charged side chain, such as arginine, lysine, aspartic acid, and glutamic acid. Any stereoisomer (i.e., L, D, or a mixture thereof) of a particular amino acid (e.g. methionine, histidine, imidazole, arginine, lysine, isoleucine, aspartic acid, tryptophan, threonine and mixtures thereof) or combinations of these stereoisomers, may be present in the pharmaceutical compositions of the invention so long as the particular amino acid is present either in its free base form or its salt form. In one embodiment the L-stereoisomer is used. Compositions of the invention may also be formulated with analogues of these amino acids. By "amino acid analogue" is intended a derivative of the naturally occurring amino acid that brings about the desired effect of decreasing aggregate formation by the polypeptide during storage of the liquid pharmaceutical compositions of the invention. Suitable arginine analogues include, for example, aminoguanidine, ornithine and N-monoethyl L-arginine, suitable methionine analogues include ethionine and buthionine and suitable cysteine analogues include S-methyl-L cysteine. As with the other amino acids, the amino acid analogues are incorporated into the compositions in either their free base form or their salt form. In a further embodiment of the invention the amino acids or amino acid analogues are used in a concentration, which is sufficient to prevent or delay aggregation of the protein.

In a further embodiment of the invention methionine (or other sulphuric amino acids or amino acid analogous) may be added to inhibit oxidation of methionine residues to methionine sulfoxide when the polypeptide acting as the therapeutic agent is a polypeptide comprising at least one methionine residue susceptible to such oxidation. By "inhibit" is intended minimal accumulation of methionine oxidized species over time. Inhibiting methionine oxidation results in greater retention of the polypeptide in its proper molecular form. Any stereoisomer of methionine (L or D) or combinations thereof can be used. The amount to be added should be an amount sufficient to inhibit oxidation of the methionine residues such that the amount of methionine sulfoxide is acceptable to regulatory agencies. Typically, this means that the composition contains no more than about 10% to about 30% methionine sulfoxide. Generally, this can be achieved by adding methionine such that the ratio of methionine added to methionine residues ranges from about 1:1 to about 1000:1, such as 10:1 to about 100:1.

In a further embodiment of the invention the formulation further comprises a stabilizer selected from the group of high molecular weight polymers or low molecular compounds. In a further embodiment of the invention the stabilizer is selected from polyethylene glycol (e.g. PEG 3350), polyvinyl alcohol (PVA), polyvinylpyrrolidone, carboxy-/hydroxycellulose or derivates thereof (e.g. HPC, HPC-SL, HPC-L and HPMC), cyclodextrins, sulphur-containing substances as monothioglycerol, thioglycolic acid and 2-methylthioethanol, and different salts (e.g. sodium chloride). Each one of these specific stabilizers constitutes an alternative embodiment of the invention.

The pharmaceutical compositions may also comprise additional stabilizing agents, which further enhance stability of a therapeutically active polypeptide therein. Stabilizing agents of particular interest to the present invention include, but are not limited to, methionine and EDTA, which protect the polypeptide against methionine oxidation, and a nonionic surfactant, which protects the polypeptide against aggregation associated with freeze-thawing or mechanical shearing.

In a further embodiment of the invention the formulation further comprises a surfactant. In another embodiment of the invention the pharmaceutical composition comprises two different surfactants. The term "Surfactant" as used herein refers to any molecules or ions that are comprised of a water-soluble (hydrophilic) part, the head, and a fat-soluble (lipophilic) segment. Surfactants accumulate preferably at interfaces, which the hydrophilic part is orientated towards the water (hydrophilic phase) and the lipophilic part towards the oil- or hydrophobic phase (i.e. glass, air, oil etc.). The concentration at which surfactants begin to form micelles is known as the critical micelle concentration or CMC. Furthermore, surfactants lower the surface tension of a liquid. Surfactants are also known as amphipathic compounds. The term "Detergent" is a synonym used for surfactants in general.

Anionic surfactants may be selected from the group of: Chenodeoxycholic acid, Chenodeoxycholic acid sodium salt, Cholic acid, Dehydrocholic acid, Deoxycholic acid, Deoxycholic acid methyl ester, Digitonin, Digitoxigenin, N,N-Dimethyldodecylamine N-oxide, Docusate sodium, Glycochenodeoxycholic acid sodium, Glycocholic acid hydrate, Glycodeoxycholic acid monohydrate, Glycodeoxycholic acid sodium salt, Glycodeoxycholic acid sodium salt, Glycolithocholic acid 3-sulfate disodium salt, Glycolithocholic acid ethyl ester, N-Lauroylsarcosine sodium salt, N-Lauroylsarcosine sodium salt, N-Lauroylsarcosine, N-Lauroylsarcosine, Lithium dodecyl sulfate, Lugol, 1-Octanesulfonic acid sodium salt, 1-Octanesulfonic acid sodium salt, Sodium 1-butanesulfonate, Sodium 1-decanesulfonate, Sodium 1-dodecanesulfonate, Sodium 1-heptanesulfonate, Sodium 1-heptanesulfonate, Sodium 1-nonanesulfonate, Sodium 1-propanesulfonate monohydrate, Sodium 2-bromoethanesulfonate, Sodium cholate hydrate, ox or sheep bile, Sodium cholate hydrate, Sodium choleate, Sodium deoxycholate, Sodium dodecyl sulfate, Sodium dodecyl sulfate, Sodium hexanesulfonate, Sodium octyl sulfate, Sodium pentanesulfonate, Sodium taurocholate, Taurochenodeoxycholic acid sodium salt, Taurodeoxycholic acid sodium salt monohydrate, Taurolithocholic acid 3-sulfate disodium salt, Tauroursodeoxycholic acid sodium salt, Trizma® dodecyl sulfate, DSS (docusate sodium, CAS registry no [577-11-7]), docusate calcium, CAS registry no [128-49-4]), docusate potassium, CAS registry no [7491-09-0]), SDS (sodium dodecyl sulfate or sodium lauryl sulfate), Dodecylphosphocholine (FOS-Choline-12), Decylphosphocholine (FOS-Choline-10), Nonylphosphocholine (FOS-Choline-9), dipalmitoyl phosphatidic acid, sodium caprylate, and/or Ursodeoxycholic acid.

Cationic surfactants may be selected from the group of: Alkyltrimethylammonium bromide

Benzalkonium chloride, Benzalkonium chloride, Benzyldimethylhexadecylammonium chloride, Benzyldimethyltetradecylammonium chloride, Benzyltrimethylammonium tetrachloroiodate, Dimethyldioctadecylammonium bromide, Dodecylethyldimethylammonium bromide, Dodecyltrimethylammonium bromide, Dodecyltrimethylammonium bromide, Ethylhexadecyldimethylammonium bromide, Hexadecyltrimethylammonium bromide, Hexadecyltrimethylammonium bromide, Polyoxyethylene(10)-N-tallow-1,3-diaminopropane, Thonzonium bromide, and/or Trimethyl (tetradecyl)ammonium bromide.

Nonionic surfactants may be selected from the group of: BigCHAP, Bis(polyethylene glycol bis[imidazoyl carbonyl]), block copolymers as polyethyleneoxide/polypropyleneoxide block copolymers such as poloxamers, poloxamer 188 and poloxamer 407, Brij® 35, Brij® 56, Brij® 72, Brij® 76, Brij® 92V, Brij® 97, Brij® 58P, Cremophor® EL, Decaethylene glycol monododecyl ether, N-Decanoyl-N-methylglucamine, n-Dodecanoyl-N-methylglucamide, alkyl-polyglucosides, ethoxylated castor oil, Heptaethylene glycol monodecyl ether, Heptaethylene glycol monododecyl ether, Heptaethylene glycol monotetradecyl ether, Hexaethylene glycol monododecyl ether, Hexaethylene glycol monohexadecyl ether, Hexaethylene glycol monooctadecyl ether, Hexaethylene glycol monotetradecyl ether, Igepal CA-630, Igepal CA-630, Methyl-6-O-(N-heptylcarbamoyl)-beta-D-glucopyranoside, Nonaethylene glycol monododecyl ether, N-Nonanoyl-N-methylglucamine, N-Nonanoyl-N-methylglucamine, Octaethylene glycol monodecyl ether, Octaethylene glycol monododecyl ether, Octaethylene glycol monohexadecyl ether, Octaethylene glycol monooctadecyl ether, Octaethylene glycol monotetradecyl ether, Octyl-β-D-glucopyranoside, Pentaethylene glycol monodecyl ether, Pentaethylene glycol monododecyl ether, Pentaethylene glycol monohexadecyl ether, Pentaethylene glycol monohexyl ether, Pentaethylene glycol monooctadecyl ether, Pentaethylene glycol monooctyl ether, Polyethylene glycol diglycidyl ether, Polyethylene glycol ether W-1, Polyoxyethylene 10 tridecyl ether, Polyoxyethylene 100 stearate, Polyoxyethylene 20 isohexadecyl ether, Polyoxyethylene 20 oleyl ether, Polyoxyethylene 40 stearate, Polyoxyethylene 50 stearate, Polyoxyethylene 8 stearate, Polyoxyethylene bis(imidazolyl carbonyl), Polyoxyethylene 25 propylene glycol stearate, Saponin from Quillaja bark, Span® 20, Span® 40, Span®60, Span® 65, Span® 80, Span® 85, Tergitol, Type 15-S-12, Tergitol, Type 15-S-30, Tergitol, Type 15-S-5, Tergitol, Type 15-S-7, Tergitol, Type 15-S-9, Tergitol, Type NP-10, Tergitol, Type NP-4, Tergitol, Type NP-40, Tergitol, Type NP-7, Tergitol, Type NP-9, Tetradecyl-β-D-maltoside, Tetraethylene glycol monodecyl ether, Tetraethylene glycol monododecyl ether, Tetraethylene glycol monotetradecyl ether, Triethylene glycol monodecyl ether, Triethylene glycol monododecyl ether, Triethylene glycol monohexadecyl ether, Triethylene glycol monooctyl ether, Triethylene glycol monotetradecyl ether, Triton CF-21, Triton CF-32, Triton DF-12, Triton DF-16, Triton GR-5M, Triton QS-15, Triton QS-44, Triton X-100, Triton X-102, Triton X-15, Triton X-151, Triton X-200, Triton X-207, Triton® X-100, Triton® X-114, Triton® X-165 solution, Triton® X-305 solution, Triton® X-405, Triton® X-45, Triton® X-705-70, TWEEN® 20, TWEEN® 40, TWEEN® 60, TWEEN® 6, TWEEN® 65, TWEEN® 80, TWEEN® 81, TWEEN® 85, Tyloxapol, sphingophospholipids (sphingomyelin), and sphingoglycolipids (ceramides, gangliosides), phospholipids, and/or n-Undecyl β-D-glucopyranoside.

Zwitterionic surfactants may be selected from the group of: CHAPS, CHAPSO, 3-(Decyldimethylammonio)propanesulfonate inner salt, 3-(Dodecyldimethylammonio)-propanesulfonate inner salt, 3-(Dodecyldimethylammonio)propanesulfonate inner salt, 3-(N,N-Dimethylmyristylammonio) propanesulfonate, 3-(N,N-Dimethyloctadecylammonio)-propanesulfonate, 3-(N,N-Dimethyloctylammonio) propanesulfonate inner salt, 3-(N,N-Dimethylpalmitylammonio)propanesulfonate, N-alkyl-N,N-dimethylammonio-1-propanesulfonates, 3-cholamido-1-propyldimethylammonio-1-propanesulfonate, Dodecylphosphocholine, myristoyl lysophosphatidylcholine, Zwittergent 3-12 (N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate), Zwittergent 3-10 (3-(Decyldimethylammonio)-propanesulfonate inner salt), Zwittergent 3-08 (3-(Octyldimethylammonio)pro-panesulfonate), glycerophospholipids (lecithins, kephalins, phosphatidyl serine), glyceroglycolipids (galactopyranoside), alkyl, alkoxyl (alkyl ester), alkoxy (alkyl ether)-derivatives of lysophosphatidyl and phosphatidylcholines, e.g. lauroyl and myristoyl derivatives of lysophosphatidylcholine, dipalmitoylphosphatidylcholine, and modifications of the polar head group, that is cholines, ethanolamines, phosphatidic acid, serines, threonines, glycerol, inositol, lysophosphatidylserine and lysophosphatidylthreonine, acylcarnitines and derivatives, N^{beta}-acylated derivatives of lysine, arginine or histidine, or side-chain acylated derivatives of lysine or arginine, N^{beta}-acylated derivatives of dipeptides comprising any combination of lysine, arginine or histidine and a neutral or acidic amino acid, N^{beta}-acylated derivative of a tripeptide comprising any combination of a neutral amino acid and two charged amino acids, or the surfactant may be selected from the group of imidazoline derivatives, long-chain fatty acids and salts thereof C_6-C_{12} (eg. oleic acid and caprylic acid), N-Hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, anionic (alkyl-aryl-sulphonates) monovalent surfactants, palmitoyl lysophosphatidyl-L-serine, lysophospholipids (e.g. 1-acyl-sn-glycero-3-phosphate esters of ethanolamine, choline, serine or threonine), or mixtures thereof.

The term "alkyl-polyglucosides" as used herein in relates to an straight or branched C_{5-20}-alkyl, -alkenyl or -alkynyl chain which is substituted by one or more glucoside moieties such as maltoside, saccharide etc. Embodiments of these alkyl-polyglucosides include C_{6-18}-alkyl-polyglucosides. Specific embodiments of these alkyl-polyglucosides includes the even numbered carbon-chains such as C_6, C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18} and C_{20} alkyl chain. Specific embodiments of the glucoside moieties include pyranoside, glucopyranoside, maltoside, maltotrioside and sucrose. In embodiments of the invention less than 6 glucosid moieties are attached to the alkyl group. In embodiments of the invention less than 5 glucosid moieties are attached to the alkyl group. In embodiments of the invention less than 4 glucosid moieties are attached to the alkyl group. In embodiments of the invention less than 3 glucosid moieties are attached to the alkyl group. In embodiments of the invention less than 2 glucosid moieties are attached to the alkyl group. Specific embodiments of alkyl-polyglucosides are alkyl glucosides such n-decyl β-D-glucopyranoside, decyl β-D-maltopyranoside, dodecyl β-D-glucopyranoside, n-dodecyl β-D-maltoside, n-dodecyl β-D-maltoside, n-dodecyl β-D-maltoside, tetradecyl β-D-glucopyranoside, decyl β-D-maltoside, hexadecyl β-D-maltoside, decyl β-D-maltotrioside, dodecyl β-D-maltotrioside, tetradecyl β-D-maltotrioside, hexadecyl β-D-maltotrioside, n-dodecyl-sucrose, n-decyl-sucrose, sucrose monocaprate, sucrose monolaurate, sucrose monomyristate, and sucrose monopalmitate.

The use of a surfactant in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: *The Science and Practice of Pharmacy,* 19th edition, 1995.

In a further embodiment of the invention the formulation further comprises protease inhibitors such as EDTA (ethylenediamine tetraacetic acid) and benzamidineHCl, but other commercially available protease inhibitors may also be used. The use of a protease inhibitor is particular useful in pharmaceutical compositions comprising zymogens of proteases in order to inhibit autocatalysis.

It is possible that other ingredients may be present in the peptide pharmaceutical formulation of the present invention. Such additional ingredients may include wetting agents, emulsifiers, antioxidants, bulking agents, tonicity modifiers, chelating agents, metal ions, oleaginous vehicles, proteins (e.g., human serum albumin, gelatine or proteins) and a zwitterion (e.g., an amino acid such as betaine, taurine, arginine, glycine, lysine and histidine). Such additional ingredients, of course, should not adversely affect the overall stability of the pharmaceutical formulation of the present invention.

Pharmaceutical compositions containing a compound according to the present invention may be administered to a patient in need of such treatment at several sites, for example, at topical sites, for example, skin and mucosal sites, at sites which bypass absorption, for example, administration in an artery, in a vein, in the heart, and at sites which involve absorption, for example, administration in the skin, under the skin, in a muscle or in the abdomen.

Administration of pharmaceutical compositions according to the invention may be through several routes of administration, for example, lingual, sublingual, buccal, in the mouth, oral, in the stomach and intestine, nasal, pulmonary, for example, through the bronchioles and alveoli or a combination thereof, epidermal, dermal, transdermal, vaginal, rectal, ocular, for examples through the conjunctiva, uretal, and parenteral to patients in need of such a treatment.

Compositions of the current invention may be administered in several dosage forms, for example, as solutions, suspensions, emulsions, microemulsions, multiple emulsion, foams, salves, pastes, plasters, ointments, tablets, coated tablets, rinses, capsules, for example, hard gelatine capsules and soft gelatine capsules, suppositories, rectal capsules, drops, gels, sprays, powder, aerosols, inhalants, eye drops, ophthalmic ointments, ophthalmic rinses, vaginal pessaries, vaginal rings, vaginal ointments, injection solution, in situ transforming solutions, for example in situ gelling, in situ setting, in situ precipitating, in situ crystallization, infusion solution, and implants.

Compositions of the invention may further be compounded in, or attached to, for example through covalent, hydrophobic and electrostatic interactions, a drug carrier, drug delivery system and advanced drug delivery system in order to further enhance stability of the compound of the present invention, increase bioavailability, increase solubility, decrease adverse effects, achieve chronotherapy well known to those skilled in the art, and increase patient compliance or any combination thereof. Examples of carriers, drug delivery systems and advanced drug delivery systems include, but are not limited to, polymers, for example cellulose and derivatives, polysaccharides, for example dextran and derivatives, starch and derivatives, poly(vinyl alcohol), acrylate and methacrylate polymers, polylactic and polyglycolic acid and block co-polymers thereof, polyethylene glycols, carrier proteins, for example albumin, gels, for example, thermogelling systems, for example block co-polymeric systems well known to those skilled in the art, micelles, liposomes, microspheres, nanoparticulates, liquid crystals and dispersions thereof, L2 phase and dispersions there of, well known to those skilled in the art of phase behaviour in lipid-water systems, polymeric micelles, multiple emulsions, self-emulsifying, self-microemulsifying, cyclodextrins and derivatives thereof, and dendrimers.

Compositions of the current invention are useful in the formulation of solids, semisolids, powder and solutions for pulmonary administration of compounds of the present invention, using, for example a metered dose inhaler, dry powder inhaler and a nebulizer, all being devices well known to those skilled in the art.

Compositions of the current invention are specifically useful in the formulation of controlled, sustained, protracting, retarded, and slow release drug delivery systems. More specifically, but not limited to, compositions are useful in formulation of parenteral controlled release and sustained release systems (both systems leading to a many-fold reduction in number of administrations), well known to those skilled in the art. Even more preferably, are controlled release and sustained release systems administered subcutaneous. Without limiting the scope of the invention, examples of useful controlled release system and compositions are hydrogels, oleaginous gels, liquid crystals, polymeric micelles, microspheres, nanoparticles, Methods to produce controlled release systems useful for compositions of the current invention include, but are not limited to, crystallization, condensation, co-crystallization, precipitation, co-precipitation, emulsification, dispersion, high pressure homogenisation, encapsulation, spray drying, microencapsulating, coacervation, phase separation, solvent evaporation to produce microspheres, extrusion and supercritical fluid processes. General reference is made to Handbook of Pharmaceutical Controlled Release (Wise, D. L., ed. Marcel Dekker, New York, 2000) and Drug and the Pharmaceutical Sciences vol. 99: Protein Formulation and Delivery (MacNally, E. J., ed. Marcel Dekker, New York, 2000).

Parenteral administration may be performed by subcutaneous, intramuscular, intraperitoneal or intravenous injection by means of a syringe, optionally a pen-like syringe. Alternatively, parenteral administration can be performed by means of an infusion pump. A further option is a composition which may be a solution or suspension or a powder for the administration of the compound of the present invention in the form of a nasal or pulmonal liquid or powder spray. As a still further option, the pharmaceutical compositions containing the compound of the invention can also be adapted to transdermal administration, e.g. by needle-free injection or from a patch, optionally an iontophoretic patch, or transmucosal, e.g. buccal, administration.

The compounds of the present invention can be administered via the pulmonary route in a vehicle, as a solution, suspension or dry powder using any of known types of devices suitable for pulmonary drug delivery. Examples of these comprise, but are not limited to, the three general types of aerosol-generating for pulmonary drug delivery, and may include jet or ultrasonic nebulizers, metered-dose inhalers, or dry powder inhalers (Cf. Yu J, Chien Y W. Pulmonary drug delivery: Physiologic and mechanistic aspects. Crit Rev Ther Drug Carr Sys 14(4) (1997) 395-453).

Based on standardised testing methodology, the aerodynamic diameter (d_a) of a particle is defined as the geometric equivalent diameter of a reference standard spherical particle of unit density (1 g/cm^3). In the simplest case, for spherical particles, d_a is related to a reference diameter (d) as a function of the square root of the density ratio as described by:

Modifications to this relationship occur for non-spherical particles (cf. Edwards D A, Ben-Jebria A, Langer R. Recent advances in pulmonary drug delivery using large, porous inhaled particles. J Appl Physiol 84(2) (1998) 379-385). The terms “MMAD” and “MMEAD” are well-described and known to the art (cf. Edwards D A, Ben-Jebria A, Langer R and represents a measure of the median value of an aerodynamic particle size distribution. Recent advances in pulmonary drug delivery using large, porous inhaled particles. J Appl Physiol 84(2) (1998) 379-385). Mass median aerody-

namic diameter (MMAD) and mass median effective aerodynamic diameter (MMEAD) are used inter-changeably, are statistical parameters, and empirically describe the size of aerosol particles in relation to their potential to deposit in the lungs, independent of actual shape, size, or density (cf. Edwards D A, Ben-Jebria A, Langer R. Recent advances in pulmonary drug delivery using large, porous inhaled particles. J Appl Physiol 84(2) (1998) 379-385). MMAD is normally calculated from the measurement made with impactors, an instrument that measures the particle inertial behaviour in air.

In a further embodiment, the formulation could be aerosolized by any known aerosolisation technology, such as nebulisation, to achieve a MMAD of aerosol particles less than 10 μm, more preferably between 1-5 μm, and most preferably between 1-3 μm. The preferred particle size is based on the most effective size for delivery of drug to the deep lung, where protein is optimally absorbed (cf. Edwards D A, Ben-Jebria A, Langer A, Recent advances in pulmonary drug delivery using large, porous inhaled particles. J Appl Physiol 84(2) (1998) 379-385).

Deep lung deposition of the pulmonal formulations comprising the compound of the present invention may optional be further optimized by using modifications of the inhalation techniques, for example, but not limited to: slow inhalation flow (eg. 30 L/min), breath holding and timing of actuation.

The term "stabilized formulation" refers to a formulation with increased physical stability, increased chemical stability or increased physical and chemical stability.

The term "physical stability" of the protein formulation as used herein refers to the tendency of the protein to form biologically inactive and/or insoluble aggregates of the protein as a result of exposure of the protein to thermo-mechanical stresses and/or interaction with interfaces and surfaces that are destabilizing, such as hydrophobic surfaces and interfaces. Physical stability of the aqueous protein formulations is evaluated by means of visual inspection and/or turbidity measurements after exposing the formulation filled in suitable containers (e.g. cartridges or vials) to mechanical/physical stress (e.g. agitation) at different temperatures for various time periods. Visual inspection of the formulations is performed in a sharp focused light with a dark background. The turbidity of the formulation is characterized by a visual score ranking the degree of turbidity for instance on a scale from 0 to 3 (a formulation showing no turbidity corresponds to a visual score 0, and a formulation showing visual turbidity in daylight corresponds to visual score 3). A formulation is classified physical unstable with respect to protein aggregation, when it shows visual turbidity in daylight. Alternatively, the turbidity of the formulation can be evaluated by simple turbidity measurements well-known to the skilled person. Physical stability of the aqueous protein formulations can also be evaluated by using a spectroscopic agent or probe of the conformational status of the protein. The probe is preferably a small molecule that preferentially binds to a non-native conformer of the protein. One example of a small molecular spectroscopic probe of protein structure is Thioflavin T. Thioflavin T is a fluorescent dye that has been widely used for the detection of amyloid fibrils. In the presence of fibrils, and perhaps other protein configurations as well, Thioflavin T gives rise to a new excitation maximum at about 450 nm and enhanced emission at about 482 nm when bound to a fibril protein form. Unbound Thioflavin T is essentially non-fluorescent at the wavelengths.

Other small molecules can be used as probes of the changes in protein structure from native to non-native states. For instance the "hydrophobic patch" probes that bind preferentially to exposed hydrophobic patches of a protein. The hydrophobic patches are generally buried within the tertiary structure of a protein in its native state, but become exposed as a protein begins to unfold or denature. Examples of these small molecular, spectroscopic probes are aromatic, hydrophobic dyes, such as antrhacene, acridine, phenanthroline or the like. Other spectroscopic probes are metal-amino acid complexes, such as cobalt metal complexes of hydrophobic amino acids, such as phenylalanine, leucine, isoleucine, methionine, and valine, or the like.

The term "chemical stability" of the protein formulation as used herein refers to chemical covalent changes in the protein structure leading to formation of chemical degradation products with potential less biological potency and/or potential increased immunogenic properties compared to the native protein structure. Various chemical degradation products can be formed depending on the type and nature of the native protein and the environment to which the protein is exposed. Elimination of chemical degradation can most probably not be completely avoided and increasing amounts of chemical degradation products is often seen during storage and use of the protein formulation as well-known by the person skilled in the art. Most proteins are prone to deamidation, a process in which the side chain amide group in glutaminyl or asparaginyl residues is hydrolysed to form a free carboxylic acid. Other degradations pathways involves formation of high molecular weight transformation products where two or more protein molecules are covalently bound to each other through transamidation and/or disulfide interactions leading to formation of covalently bound dimer, oligomer and polymer degradation products (*Stability of Protein Pharmaceuticals, Ahern*. T. J. & Manning M. C., Plenum Press, New York 1992). Oxidation (of for instance methionine residues) can be mentioned as another variant of chemical degradation. The chemical stability of the protein formulation can be evaluated by measuring the amount of the chemical degradation products at various time-points after exposure to different environmental conditions (the formation of degradation products can often be accelerated by for instance increasing temperature). The amount of each individual degradation product is often determined by separation of the degradation products depending on molecule size and/or charge using various chromatography techniques (e.g. SEC-HPLC and/or RP-HPLC).

Hence, as outlined above, a "stabilized formulation" refers to a formulation with increased physical stability, increased chemical stability or increased physical and chemical stability. In general, a formulation must be stable during use and storage (in compliance with recommended use and storage conditions) until the expiration date is reached.

In one embodiment of the invention the pharmaceutical formulation comprising the compound of the present invention is stable for more than 6 weeks of usage and for more than 3 years of storage.

In another embodiment of the invention the pharmaceutical formulation comprising the compound of the present invention is stable for more than 4 weeks of usage and for more than 3 years of storage.

In a further embodiment of the invention the pharmaceutical formulation comprising the compound of the present invention is stable for more than 4 weeks of usage and for more than two years of storage.

In an even further embodiment of the invention the pharmaceutical formulation comprising the compound of the present invention is stable for more than 2 weeks of usage and for more than two years of storage.

In another aspect the present invention relates to the use of a compound according to the invention for the preparation of a medicament.

In one embodiment a compound according to the invention is used for the preparation of a medicament for the treatment or prevention of hyperglycemia, type 2 diabetes, impaired glucose tolerance, type 1 diabetes, obesity, hypertension, syndrome X, dyslipidemia, cognitive disorders, atheroschlerosis, myocardial infarction, stroke, coronary heart disease and other cardiovascular disorders, inflammatory bowel syndrome, dyspepsia and gastric ulcers.

In another embodiment a compound according to the invention is used for the preparation of a medicament for delaying or preventing disease progression in type 2 diabetes.

In another embodiment a compound according to the invention is used for the preparation of a medicament for decreasing food intake, decreasing β-cell apoptosis, increasing β-cell function and β-cell mass, and/or for restoring glucose sensitivity to β-cells.

The treatment with a compound according to the present invention may also be combined with a second or more pharmacologically active substances, e.g. selected from antidiabetic agents, antiobesity agents, appetite regulating agents, antihypertensive agents, agents for the treatment and/or prevention of complications resulting from or associated with diabetes and agents for the treatment and/or prevention of complications and disorders resulting from or associated with obesity. Examples of these pharmacologically active substances are: Insulin, sulphonylureas, biguanides, meglitinides, glucosidase inhibitors, glucagon antagonists, DPP-IV (dipeptidyl peptidase-IV) inhibitors, inhibitors of hepatic enzymes involved in stimulation of gluconeogenesis and/or glycogenolysis, glucose uptake modulators, compounds modifying the lipid metabolism such as antihyperlipidemic agents as HMG CoA inhibitors (statins), Gastric Inhibitory Polypeptides (GIP analogs), compounds lowering food intake, RXR agonists and agents acting on the ATP-dependent potassium channel of the β-cells; Cholestyramine, colestipol, clofibrate, gemfibrozil, lovastatin, pravastatin, simvastatin, probucol, dextrothyroxine, neteglinide, repaglinide; β-blockers such as alprenolol, atenolol, timolol, pindolol, propranolol and metoprolol, ACE (angiotensin converting enzyme) inhibitors such as benazepril, captopril, enalapril, fosinopril, lisinopril, alatriopril, quinapril and ramipril, calcium channel blockers such as nifedipine, felodipine, nicardipine, isradipine, nimodipine, diltiazem and verapamil, and β-blockers such as doxazosin, urapidil, prazosin and terazosin; CART (cocaine amphetamine regulated transcript) agonists, NPY (neuropeptide Y) antagonists, PYY agonist, PYY2 agonists, PYY4 agonists, mixed PPY2/PYY4 agonists, MC4 (melanocortin 4) agonists, orexin antagonists, TNF (tumor necrosis factor) agonists, CRF (corticotropin releasing factor) agonists, CRF BP (corticotropin releasing factor binding protein) antagonists, urocortin agonists, β3 agonists, MSH (melanocyte-stimulating hormone) agonists, MCH (melanocyte-concentrating hormone) antagonists, CCK (cholecystokinin) agonists, serotonin re-uptake inhibitors, serotonin and noradrenaline re-uptake inhibitors, mixed serotonin and noradrenergic compounds, 5HT (serotonin) agonists, bombesin agonists, galanin antagonists, growth hormone, growth hormone releasing compounds, TRH (thyreotropin releasing hormone) agonists, UCP 2 or 3 (uncoupling protein 2 or 3) modulators, leptin agonists, DA agonists (bromocriptin, doprexin), lipase/amylase inhibitors, RXR (retinoid X receptor) modulators, TR β agonists; histamine H3 antagonists, Gastric Inhibitory Polypeptide agonists or antagonists (GIP analogs), gastrin and gastrin analogs.

The treatment with a compound according to this invention may also be combined with surgery—a surgery that influence the glucose levels and/or lipid homeostasis such as gastric banding or gastric bypass.

It should be understood that any suitable combination of the compounds according to the invention with one or more of the above-mentioned compounds and optionally one or more further pharmacologically active substances are considered to be within the scope of the present invention.

The present invention is further illustrated by the following examples which, however, are not to be construed as limiting the scope of protection. The features disclosed in the foregoing description and in the following examples may, both separately and in any combination thereof, be material for realising the invention in diverse forms thereof.

EXAMPLES

Abbreviations Used:

r.t: Room temperature
DIPEA: diisopropylethylamine
H_2O: water
CH_3CN: acetonitrile
DMF: NN dimethylformamide
HBTU: 2-(1H-Benzotriazol-1-yl-)-1,1,3,3 tetramethyluronium hexafluorophosphate
Fmoc: 9H-fluoren-9-ylmethoxycarbonyl
Boc: tert butyloxycarbonyl
OtBu: tert butyl ester
tBu: tert butyl
Trt: triphenylmethyl
Pmc: 2,2,5,7,8-Pentamethyl-chroman-6-sulfonyl
Dde: 1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)ethyl
ivDde: 1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl
Mtt: 4-methyltrityl
Mmt: 4-methoxytrityl
DCM: dichloromethane
TIS: triisopropylsilane)
TFA: trifluoroacetic acid
Et_2O: diethylether
NMP: 1-Methyl-pyrrolidin-2-one
DIPEA: Diisopropylethylamine
HOAt: 1-Hydroxy-7-azabenzotriazole
HOBt: 1-Hydroxybenzotriazole
DIC: Diisopropylcarbodiimide

A: Synthesis of Resin Bound Peptide.

The protected peptidyl resin was synthesized according to the Fmoc strategy on an Applied Biosystems 433 peptide synthesizer in 0.25 mmol or 1.0 mmol scale using the manufacturer supplied FastMoc UV protocols which employ HBTU (2-(1H-Benzotriazol-1-yl-)-1,1,3,3 tetramethyluronium hexafluorophosphate) or HATU (O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) mediated couplings in NMP (N-methyl pyrrolidone), and UV monitoring of the deprotection of the Fmoc protection group. The starting resin used for the synthesis of the GLP-1 peptide amides was Rink-Amide resin and either Wang or chlorotrityl resin was used for GLP-1 peptides with a carboxy C-terminal. The protected amino acid derivatives used were standard Fmoc-amino acids (supplied from e.g. Anaspec, or Novabiochem) supplied in preweighed cartridges suitable for the ABI433A synthesizer with the exception of unnatural aminoacids such as Fmoc-Aib-OH (Fmoc-aminoisobutyric acid). The N terminal amino acid was Boc protected at the alpha amino group (e.g. Boc-His(Boc)OH was used for peptides with His at the N-terminal). The epsilon amino group of

lysine in position 26 was either protected with Mtt, Mmt, Dde, ivDde, or Boc, depending on the route for attachment of the albumin binding moiety and spacer. The synthesis of the peptides may in some cases be improved by the use of dipeptides protected on the dipeptide amide bond with a group that can be cleaved under acidic conditions such but not limited to 2-Fmoc-oxy-4-methoxybenzyl or 2,4,6-trimethoxybenzyl. In cases where a serine or a threonine is present in the peptide, the use of pseudoproline dipeptides may be used (see e.g. catalogue from Novobiochem 2002/2003 or newer version, or W. R. Sampson (1999), J. Pep. Sci. 5, 403.

Procedure for Removal of ivDde or Dde-Protection.

The resin (0.25 mmol) was placed in a manual shaker/filtration apparatus and treated with 2% hydrazine in N-methylpyrrolidone (20 ml, 2×12 min) to remove the Dde or ivDde group and wash with N-methylpyrrolidone (4×20 ml).

Procedure for Removal of Mtt or Mmt-Protection.

The resin (0.25 mmol) was placed in a manual shaker/filtration apparatus and treated with 2% TFA and 2-3% TIS in DCM (20 ml, 5-10 min repeated 6-12 times) to remove the Mtt or Mmt group and wash with DCM (2×20 ml), 10% MeOH and 5% DIPEA in DCM (2×20 ml) and N-methylpyrrolidone (4×20 ml).

Procedure for Attachment of Sidechains to Lysine Residue.

The albumin binding residue (B—U— sidechain of formula I) can be attached to the GLP-1 peptide either by acylation to resin bound peptide or acylation in solution to the unprotected peptide using standard acylating reagent such as but not limited to DIC, HOBt/DIC, HOAt/DIC, or HBTU.

Attachment to Resin Bound Peptide:

Route I

Activated (active ester or symmetric anhydride) albumin binding residue (B—U— sidechain of formula I) such as octadecanedioic acid mono-(2,5-dioxo-pyrrolidin-1-yl) ester (Ebashi et al. EP511600, 4 molar equivalents relative to resin bound peptide) was dissolved in NMP (25 mL), added to the resin and shaken overnight at room temperature. The reaction mixture was filtered and the resin was washed extensively with NMP, dichloromethane, 2-propanol, methanol and diethyl ether.

Route II

The albumin binding residue (B—U— sidechain of formula I) was dissolved in N-methyl pyrrolidone/methylene chloride (1:1, 10 ml). The activating reagent such as hydroxybenzotriazole (HOBt) (4 molar equivalents relative to resin) and diisopropylcarbodiimide (4 molar equivalents relative to resin) was added and the solution was stirred for 15 min. The solution was added to the resin and diisopropyethylamine (4 molar equivalents relative to resin) was added. The resin was shaken 2 to 24 hours at room temperature. The resin was washed with N-methylpyrrolidone (2×20 ml), N-methyl pyrrolidone/Methylene chloride (1:1) (2×20 ml) and methylene chloride (2×20 ml).

Route III

Activated (active ester or symmetric anhydride) albumin binding residue (B—U— sidechain of formula I) such as octadecanedioic acid mono-(2,5-dioxo-pyrrolidin-1-yl) ester (Ebashi et al. EP511600, 1-1.5 molar equivalents relative to the GLP-1 peptide was dissolved in an organic solvent such as acetonitrile, THF, DMF, DMSO or in a mixture of water/organic solvent (1-2 ml) and added to a solution of the peptide in water (10-20 ml) together with 10 molar equivalents of DIPEA. In case of protecting groups on the albumin binding residue such as tert.-butyl, the reaction mixture was lyophilized O/N and the isolated crude peptide deprotected afterwards—in case of a tert-butyl group the peptide was dissolved in a mixture of trifluoroacetic acid, water and triisopropylsilane (90:5:5). After for 30 min the mixture was, evaporated in vacuo and the finale peptide purified by preparative HPLC.

Procedure for removal of Fmoc-protection: The resin (0.25 mmol) was placed in a filter flask in a manual shaking apparatus and treated with N-methylpyrrolidone/methylene:chloride (1:1) (2×20 ml) and with N-methylpyrrolidone (1×20 ml), a solution of 20% piperidine in N-methylpyrrolidone (3×20 ml, 10 min each). The resin was washed with N-methyl pyrrolidone (2×20 ml), N-methylpyrrolidone/Methylene chloride (1:1) (2×20 ml) and methylene chloride (2×20 ml).

Procedure for Cleaving the Peptide Off the Resin:

The peptide was cleaved from the resin by stirring for 180 min at room temperature with a mixture of trifluoroacetic acid, water and triisopropylsilane (95:2.5:2.5 to 92:4:4). The cleavage mixture was filtered and the filtrate was concentrated to an oil by a stream of nitrogen. The crude peptide was precipitated from this oil with 45 ml diethyl ether and washed 1 to 3 times with 45 ml diethyl ether.

Purification: The crude peptide was purified by semi-preparative HPLC on a 20 mm×250 mm column packed with either 5μ or 7μ C-18 silica. Depending on the peptide one or two purification systems were used.

TFA: After drying the crude peptide was dissolved in 5 ml 50% acetic acid H_2O and diluted to 20 ml with H_2O and injected on the column which then was eluted with a gradient of 40-60% CH_3CN in 0.1% TFA 10 ml/min during 50 min at 40° C. The peptide containing fractions were collected. The purified peptide was lyophilized after dilution of the eluate with water.

Ammonium sulphate: The column was equilibrated with 40% CH_3CN in 0.05M $(NH_4)_2SO_4$, which was adjusted to pH 2.5 with concentrated H_2SO_4. After drying the crude peptide was dissolved in 5 ml 50% acetic acid H_2O and diluted to 20 ml with H_2O and injected on the column which then was eluted with a gradient of 40%-60% CH_3CN in 0.05M $(NH_4)_2SO_4$, pH 2.5 at 10 ml/min during 50 min at 40° C. The peptide containing fractions were collected and diluted with 3 volumes of H_2O and passed through a Sep-Pak® C18 cartridge (Waters part. #: 51910) which has been equilibrated with 0.1% TFA. It was then eluted with 70% CH_3CN containing 0.1% TFA and the purified peptide was isolated by lyophilisation after dilution of the eluate with water.

The final product obtained was characterised by analytical RP-HPLC (retention time) and by LCMS

The RP-HPLC analysis was performed using UV detection at 214 nm and a Vydac 218TP54 4.6 mm×250 mm 5μ C-18 silica column (The Separations Group, Hesperia, USA) which was eluted at 1 ml/min at 42° C. Two different elution conditions were used:

A1: Equilibration of the column with in a buffer consisting of 0.1M $(NH_4)_2SO_4$, which was adjusted to pH 2.5 with concentrated H_2SO_4 and elution by a gradient of 0% to 60% CH_3CN in the same buffer during 50 min.

B1: Equilibration of the column with 0.1% TFA/H_2O and elution by a gradient of 0% CH_3CN/0.1% TFA/H_2O to 60% CH_3CN/0.1% TFA/H_2O during 50 min.

B6: Equilibration of the column with 0.1% TFA/H_2O and elution by a gradient of 0% CH_3CN/0.1% TFA/H_2O to 90% CH_3CN/0.1% TFA/H_2O during 50 min.

Alternative the RP-HPLC analysis was performed using UV detection at 214 nm and a Symmetry 300, 3.6 mm×150 mm, 3.5% C-18 silica column (Waters) which was eluted at 1 ml/min at 42° C.

B4: Equilibration of the column with 0.05% TFA/H_2O and elution by a gradient of 5% CH_3CN/0.05% TFA/H_2O to 95% CH_3CN/0.05% TFA/H_2O during 15 min.

The following instrumentation was used:

LCMS was performed on a setup consisting of Sciex API 100 Single quadropole mass spectrometer, Perkin Elmer Series 200 Quard pump, Perkin Elmer Series 200 autosampler, Applied Biosystems 785A UV detector, Sedex 75 evaporative light scattering detector

The instrument control and data acquisition were done by the Sciex Sample control software running on a Windows 2000 computer.

The HPLC pump is connected to two eluent reservoirs containing:

A: 0.05% Trifluoro acetic acid in water

B: 0.05% Trifluoro acetic acid in acetonitrile

The analysis is performed at room temperature by injecting an appropriate volume of the sample (preferably 20 μl) onto the column which is eluted with a gradient of acetonitrile.

The HPLC conditions, detector settings and mass spectrometer settings used are giving in the following table.

Column: Waters Xterra MS C-18×3 mm id 5 μm

Gradient: 5%-90% acetonitrile linear during 7.5 min at 1.5 ml/min

Detection: 210 nm (analogue output from DAD)

ELS (analogue output from ELS), 40° C.

MS ionisation mode API-ES

Alternatively LCMS was performed on a setup consisting of Hewlett Packard series 1100 G1312A Bin Pump, Hewlett Packard series 1100 Column compartment, Hewlett Packard series 1100 G1315A DAD diode array detector, Hewlett Packard series 1100 MSD and Sedere 75 Evaporative Light Scattering detector controlled by HP Chemstation software.

The HPLC pump is connected to two eluent reservoirs containing:

A: 10 mM NH_4OH in water

B: 10 mM NH_4OH in 90% acetonitrile

The analysis was performed at 23° C. by injecting an appropriate volume of the sample (preferably 20 μl) onto the column which is eluted with a gradient of A and B.

The HPLC conditions, detector settings and mass spectrometer settings used are giving in the following table.

Column Waters Xterra MS C-18×3 mm id 5 m

Gradient 5%-100% acetonitrile linear during 6.5 min at 1.5 ml/min

Detection 210 nm (analogue output from DAD)

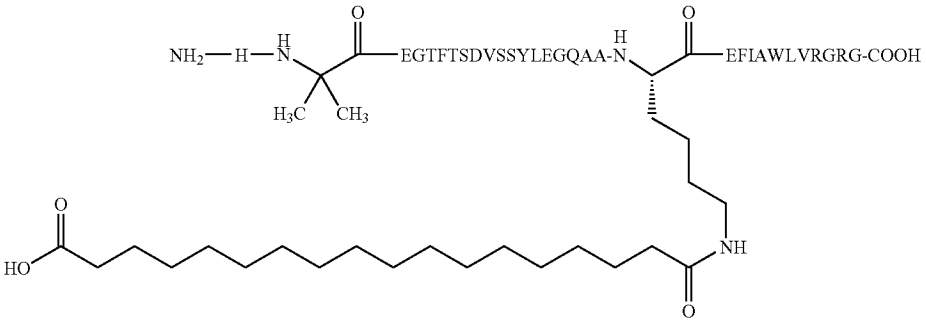
ELS (analogue output from ELS)

MS ionisation mode API-ES. Scan 100-1000 amu step 0.1 amu

Radioligand Binding to Plasma Membranes Expressing the Human GLP-1 Receptor

The binding assay was performed with purified plasma membranes containing the human GLP-1 receptor. The plasma membranes containing the receptors were purified from stably expressing BHK tk-ts 13 cells. The membranes were diluted in Assay Buffer (50 mM HEPES, 5 mM EGTA, 5 mM $MgCl_2$, 0.005% Tween 20, pH=7.4) to a final concentration of 0.2 mg/ml of protein and distributed to 96-well microtiter plates precoated with 0.3% PEI. Membranes in the presence of 0.05 nM [^{125}I]GLP-1, unlabelled ligands in increasing concentrations and different HSA concentrations (0.005%, 0.05%, and 2%) were incubated 2 hr at 30° C. After incubation, unbound ligands were separated from bound ligands by filtration through a vacuum-manifold followed by 2×100 μl washing with ice cold assay buffer. The filters were dried overnight at RT, punched out and quantified in a γ-counter.

Example 1



N-ϵ^{26} (17-carboxyheptadecanoyl)-[Aib8,Arg34] GLP-1-(7-37)-peptide

A resin (Fmoc-Gly-NovaSyn TGT, 0.22 mmol/g Novabiochem 0.25 mmole) was used to produce the primary sequence on an ABI433A machine according to manufacturers guidelines. All protecting groups were acid labile with the exception of the residue used in position 26 (FmocLys(ivDde)-OH, Novabiochem) allowing specific deprotection of this lysine rather than any other lysine.

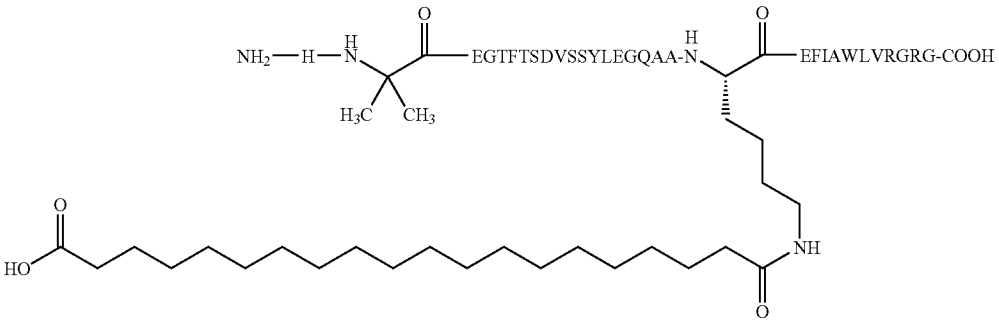
Procedure

The resin (0.09 mmole) was placed in a manual shaker/filtration apparatus and treated with 4% hydrazine in N-methylpyrrolidone in (4×10 min. 4×4 ml) to remove the ivDde group. The resin was washed with N-methylpyrrolidone (3×4 ml). Octadecanedioic acid mono-(2,5-dioxo-pyrrolidone-1-yl)ester) (4 molar equivalents relative to resin) was dissolved in DMF (4 ml). The solution was added to the resin and diisopropylethylamine (8 molar equivalents relative to resin) was added. The resin was shaken 24 hours at room temperature. The resin was washed with N-methylpyrrolidone (4×4 ml) and DCM (4×4 ml). The peptide was cleaved from the resin by stirring for 180 min at room temperature with a mixture of trifluoroacetic acid, water and triisopropylsilane (92.5:5.0:2.5 4 ml). The cleavage mixture was filtered and the crude peptide was precipitated from 40 ml diethyl ether and washed 3 times with 45 ml diethyl ether. The crude peptide was purified by preparative HPLC on a 20 mm×250 mm column packed with 7μ C-18 silica. The crude peptide was dissolved in 5 ml 50% acetic acid in water and diluted to 20 ml with H_2O and injected on the column which then was eluted with a gradient of 25-65% (CH_3CN in water with 0.1% TFA) 20 ml/min during 40 min at RT. The peptide containing fractions were collected. The purified peptide was lyophilized after dilution of the eluate with water.

HPLC (method B4): RT=9.94 min (91%)

LCMS: m/z=1232 (MH_3^{3+}) Calculated for (MH_3^{3+})=1232

Example 2



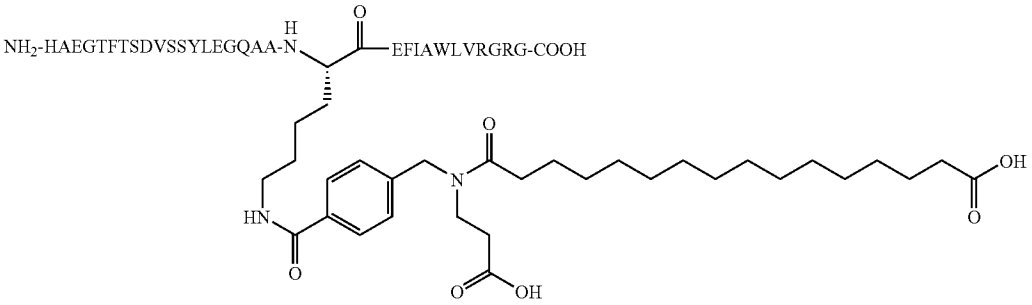
N-ε^{26}-(19-carboxynonadecanoyl)-[Aib8,Arg34] GLP-1-(7-37)-peptide

Prepared as in Example 1 and in accordance with "synthetic methods".

HPLC (method B4): RT=10.42 min (91%)

LCMS: m/z=1242 (MH_3^{3+}), Calculated for (MH_3^{3+})= 1242

Example 3



N-ε^{26}-(4-{[N-(2-carboxyethyl)-N-(15-carboxypentadecanoyl)amino]methyl}benzoyl[Arg34]GLP-1-(7-37)-peptide

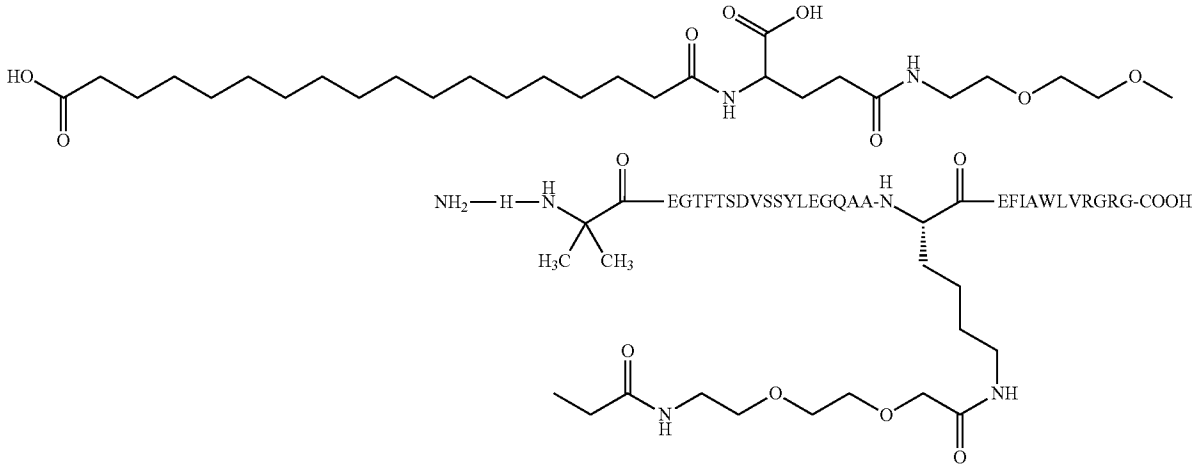
To a solution of 4-(N-(2-(tert-butoxycarbonyl)ethyl)-N-(15-(tert-butoxycarbonyl)pentadecanoyl)aminomethyl)benzoic acid (36 mg, 60 μmol) in THF (1 ml) were added DIPEA (7 μl) and O-(1-succinimidyl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (TSTU, 17 mg, 56 μl). After stirring for 1 h at room temperature, the mixture was diluted with THF (1 ml), and 1 ml of the resulting solution was added to a solution of [Arg34]GLP-1-(7-37) peptide (approx 100 mg) and DIPEA (103 μl) in water (5 ml). After 0.5 h more of the THF-solution of acylating agent (0.4 ml) was added. After stirring at room temperature for a total of 1.5 h the reaction mixture was filtered and applied to a preparative HPLC (gradient elution with 35-55% MeCN/55-35% water/10% water with 1% TFA). Fractions containing the desired product were combined and lyophylized. The product was then treated with 25 ml of a mixture of TFA and water (95/5 vol) for 15 min at room temperature, concentrated, and purified once more by HPLC. 15.4 mg of the title compound was obtained.

HPLC (method B4): RT=9.41 min (99%)

LCMS: m/z=1287 (MH_3^{3+}). Calculated for (MH_3^{3+}): 1287

Example 4

N-ϵ^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8, Arg34]GLP-1-(7-37)peptide



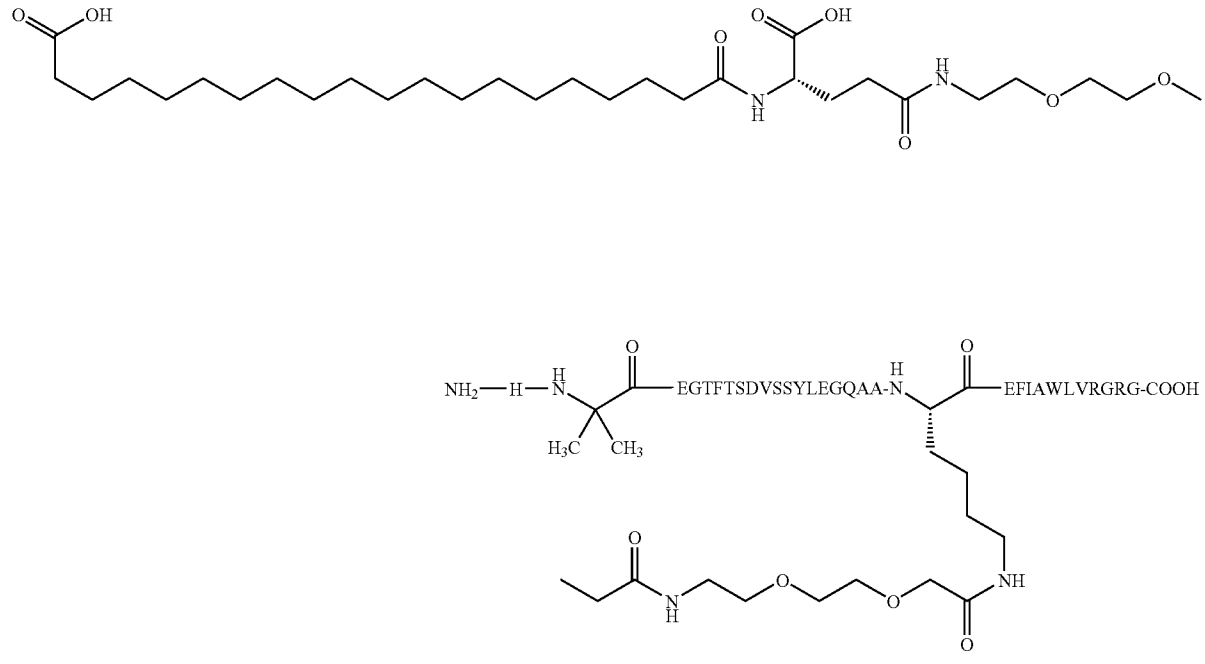
[Aib8,Arg34]GLP-1-(7-37)-peptide was prepared by standard Fmoc-solid phase peptide synthesis and purified by preparative HPLC. [Aib8,Arg34]GLP-1-(7-37)-peptide was dissolved in water (15 ml) and DIPEA (50 ul) was added. 17-((S)-1-tert-Butoxycarbonyl-3-{2-[2-({2-[2-(2,5-dioxopyrrolidin-1-yloxycarbonylmethoxy)ethoxy]ethylcarbamoyl}methoxy)ethoxy]ethylcarbamoyl}propylcarbamoyl)heptadecanoic acid tert-butyl ester (21 mg) was dissolved in acetonitrile/water 2:1 (1.5 ml) and added in small portions. The reaction was monitored by HPLC. When no more [Aib8,Arg34]GLP-1-(7-37)-peptide was found the reaction mixture was lyophilized O/N.

To the isolated compound was added 10 ml of 90% TFA/5% TIS/5% water and the reaction mixture was standing for 2 hours, evaporated in vacuo, and co-evaporated with heptane. The residual oil was dissolved in 15 ml of water containing 1% of NH3-aq and purified by preparative HPLC to give the title compound.

HPLC (method B4): RT=9.60 min (100%)

LCMS: m/z=1372 (MH_3^{3+}). Calculated for (MH_3^{3+}): 1372

Example 5



N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(19-Carboxynonadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8, Arg34]GLP-1-(7-37)peptide

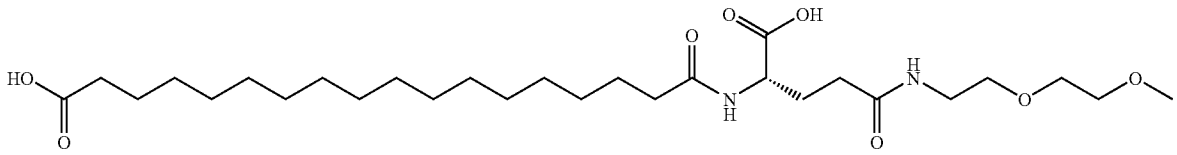
The peptide was prepared according to: A. Synthesis of resin bound peptide in 0.25 mMol scale on a Fmoc-Gly-Wang resin (0.66 mmol/g Novabiochem) was used to produce the primary sequence on an ABI433A machine according to manufacturers guidelines. All protecting groups were acid labile with the exception of the residue used in position 26 (FmocLys(Mtt)-OH, Novabiochem) which is super acid labile, allowing specific deprotection of this lysine rather than any other lysine.

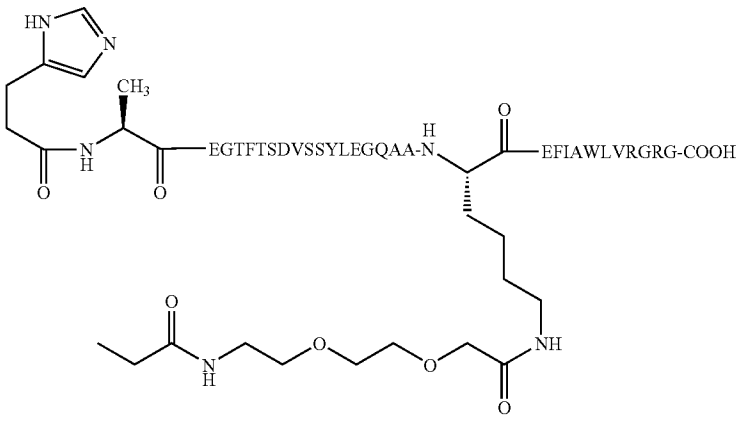
Procedure for removal of Mtt-protection. The resin (0.25 mmol) was placed in a manual shaker/filtration apparatus and treated with 2% TFA, 3% TIS in DCM (20 ml, 5-10 min repeated 6-12 times) to remove the Mtt group and wash with DMF. Synthesis was continued with Procedure for attachment of sidechains to Lysine residue, following Route II, with the appropriate Procedure for removal of Fmoc-protection. Final deprotection, HPLC-purification and analysis by HPLC and LC-MS according to the procedures.

HPLC (method B6): RT=34.56 min (100%)

LCMS: m/z=1381.8 (MH_3^{3+}). Calculated for ($M+H^+$): 4142.7

Example 6





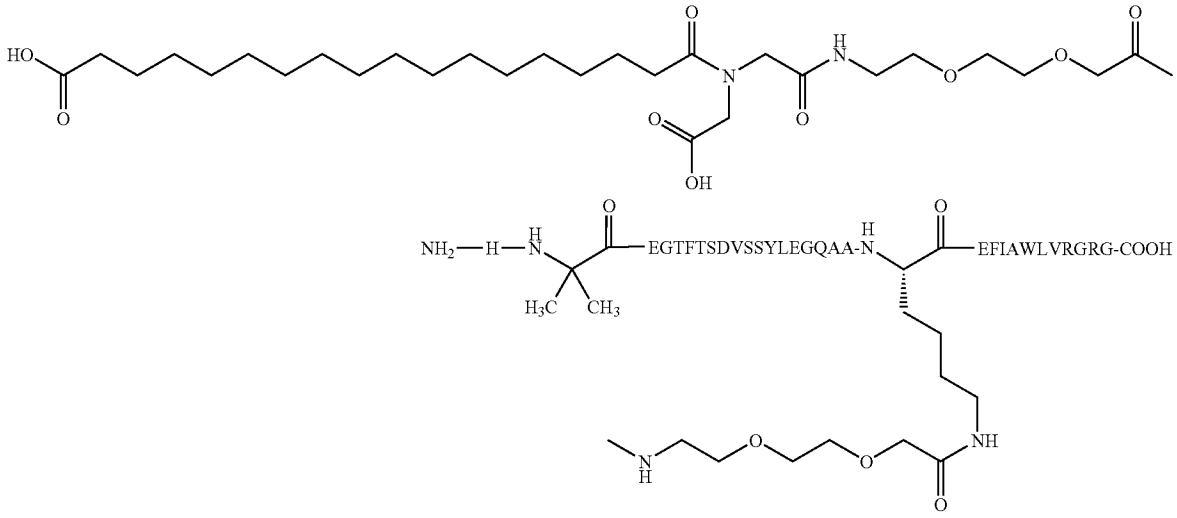
N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][3-(4-Imidazolyl)Propionyl7, Arg34]GLP-1-(7-37)peptide

Prepared as in Example 5 and in accordance with "synthetic methods".

HPLC (method B6): RT=32.89 min (100%)

LCMS: m/z=1362.3 (MH_3^{3+}). Calculated for ($M+H^+$): 4085.6

Example 7



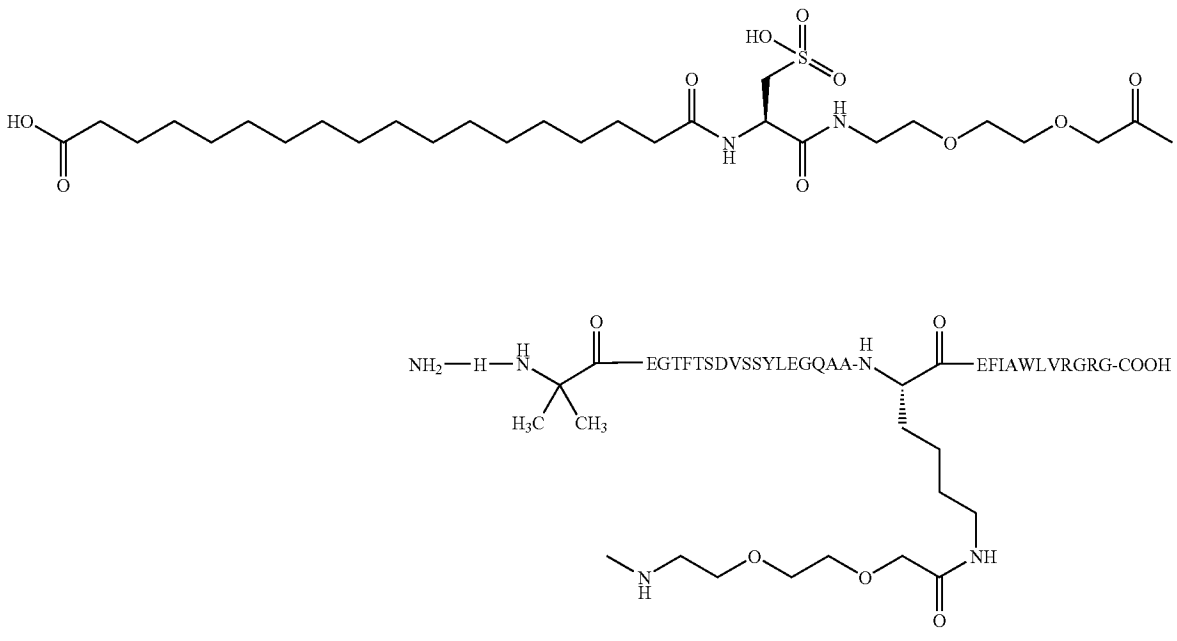
N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-(Carboxymethyl-amino)acetylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide

Prepared as in Example 5 and in accordance with "synthetic methods".

HPLC (method B6): RT=32.67 min (100%)

LCMS: m/z=1367.3 (MH_3^{3+}). Calculated for ($M+H^+$): 4100.6

Example 8



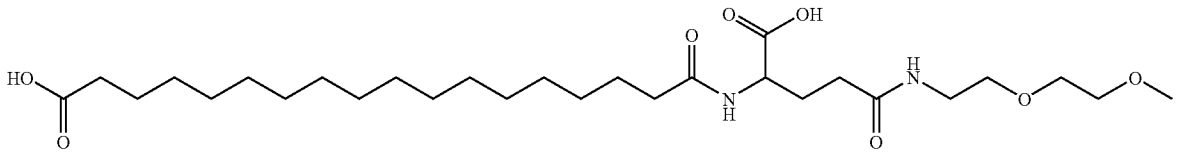
N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-3(S)-Sulfopropionylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8, Arg34]GLP-1-(7-37)peptide

Prepared as in Example 5 and in accordance with "synthetic methods".

HPLC (method B6): RT=32.04 min (100%)

LCMS: m/z=1379.8 (MH_3^{3+}). Calculated for ($M+H^+$): 4136.7

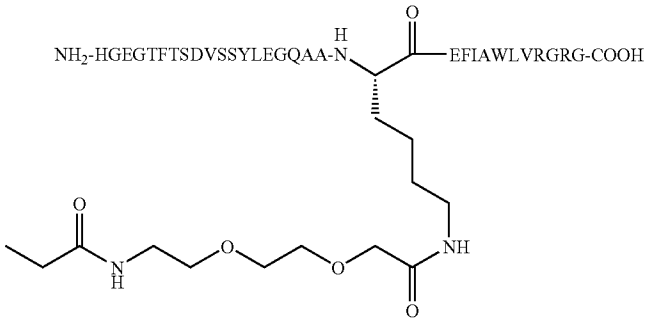
Example 9



N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Gly8, Arg34]GLP-1-(7-37)peptide

[Gly8,Arg34]GLP-1(7-37) peptide starting from 150 mg 2-chlorotrityl chloride resin (1.4 mmol/g) was prepared by

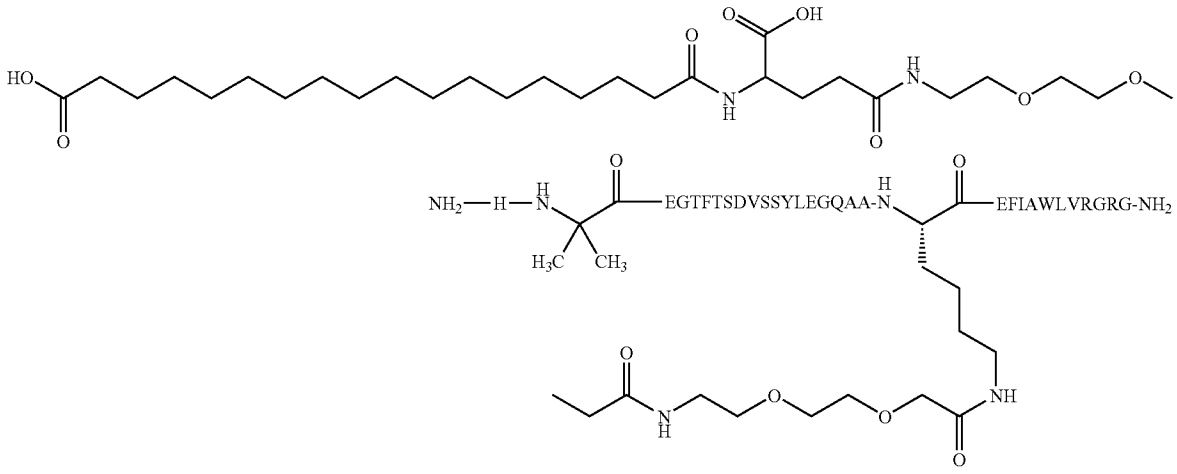
Fmoc-solid phase peptide synthesis using Apex396 from Advanced Chemtech. The Lys residue at position 26 was protected as Lys(ivDde) while the functional side-chains for the other amino acids were protected with standard acid labile protecting groups. The Lys residue was deprotected with 3% hydrazine/3% piperidine in NMP for 1 hr. Then, the two units of 8-amino-3,6-dioxaoctanoic acid, γ-glutamic acid and octadecanedioic acid were coupled to the resin attached peptide using DIC/HOAt. The peptide was finally deprotected and cleaved from the resin with TFA/TIS/H_2O/thioanisol (90/5/3/2). The peptide was isolated by LC-MS.



HPLC: Elutes at 46% acetonitrile

MALDI: 4087 (MH+)

Example 10



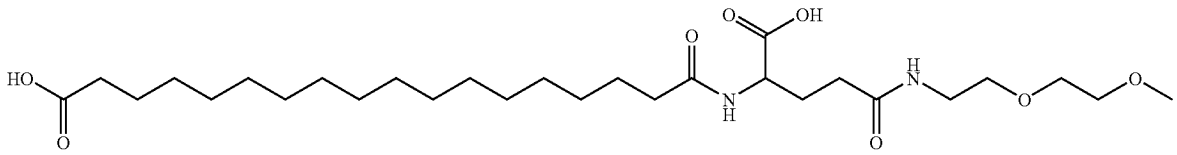
N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)-amide

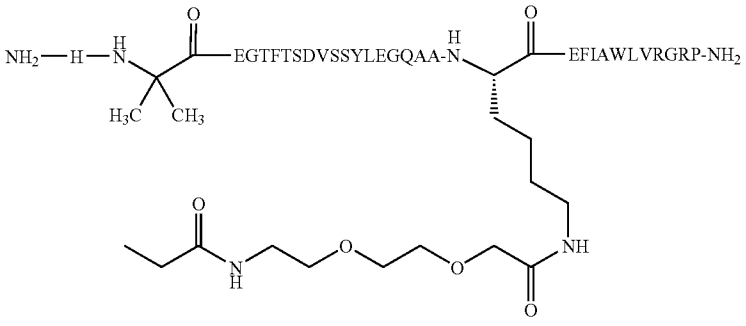
[Aib8,34]GLP-1(7-37) amide starting from 200 mg Tentagel RAM S resin (0.26 mmol/g) was prepared by Fmoc-solid phase peptide synthesis using Apex396 from Advanced Chemtech. The Lys residue at position 26 was protected as Lys(ivDde) while the functional side-chains for the other amino acids were protected with standard acid labile protecting groups. The Lys residue was deprotected with 3% hydrazine/3% piperidine in NMP for 1 hr. Then, the two units of, 8-amino-3,6-dioxaoctanoic acid, γ-glutamic acid octadecanedioic acid were coupled to the resin attached peptide using DIC/HOAt. The peptide was finally deprotected and cleaved from the resin with TFA/TIS/H_2O/thioanisol (90/5/3/2). The peptide was isolated by LC-MS.

HPLC: Elutes at 49% acetonitrile

MALDI: 4114 (MH+)

Example 11





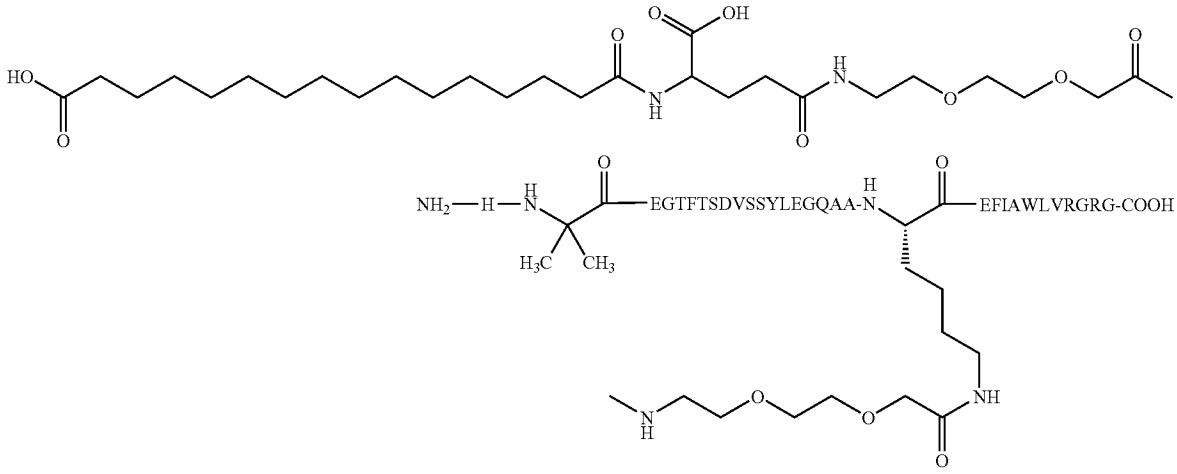
N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8, Arg34,Pro37]GLP-1-(7-37)amide

The peptide was prepared on a Rink amide resin (0.70 mmol/g Novabiochem) and else as in Example 5 and in accordance with "synthetic methods".

HPLC (method B6): RT=32.13 min (100%). (method A1): RT=44.33 min (98.4%)

LCMS: m/z=1385.3 (MH_3^{3+}). Calculated for ($M+H^+$): 4153.8

Example 12

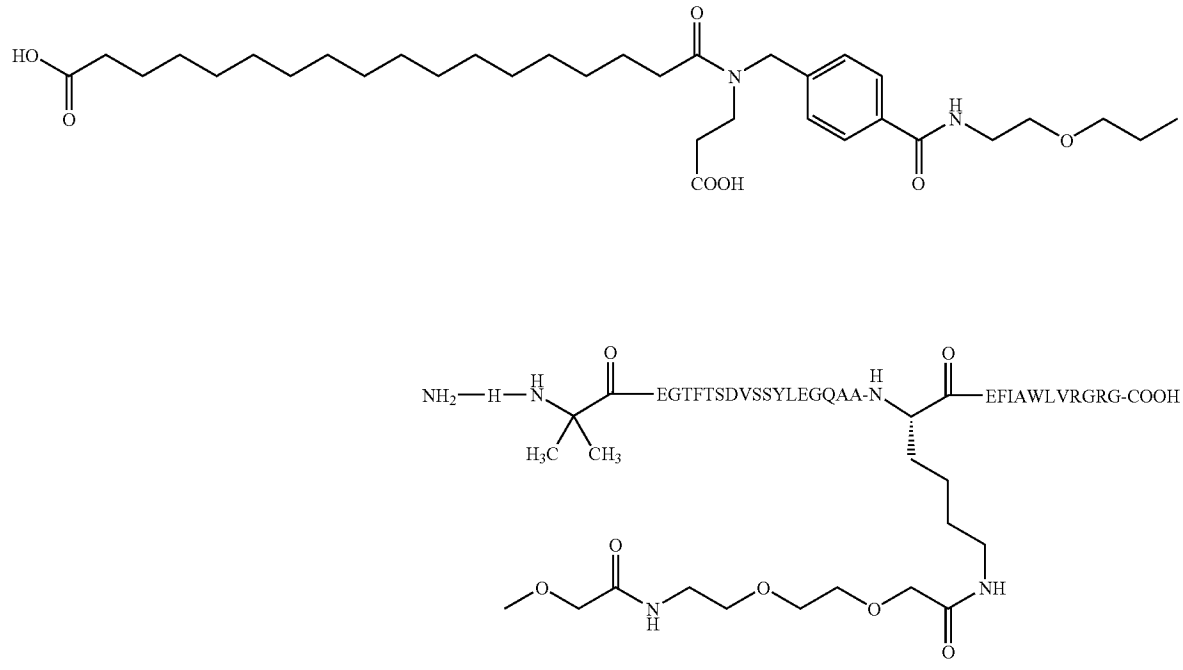


-

Aib^8, Lys^{26}(N-ε^{26}-{2-(2-(2-(2-[2-(2-(4-(pentadecanoylamino)-4-carboxybutyrylamino)ethoxy)ethoxy]acetyl)ethoxy)ethoxy)acetyl)}),Arg^{34})GLP-1H(7-37)-OH

HPLC (method B6): RT=30.41 min
LCMS: m/z=1362.9 (MH_3^{3+}) Calculated for (M^+)=4085.61

Example 13



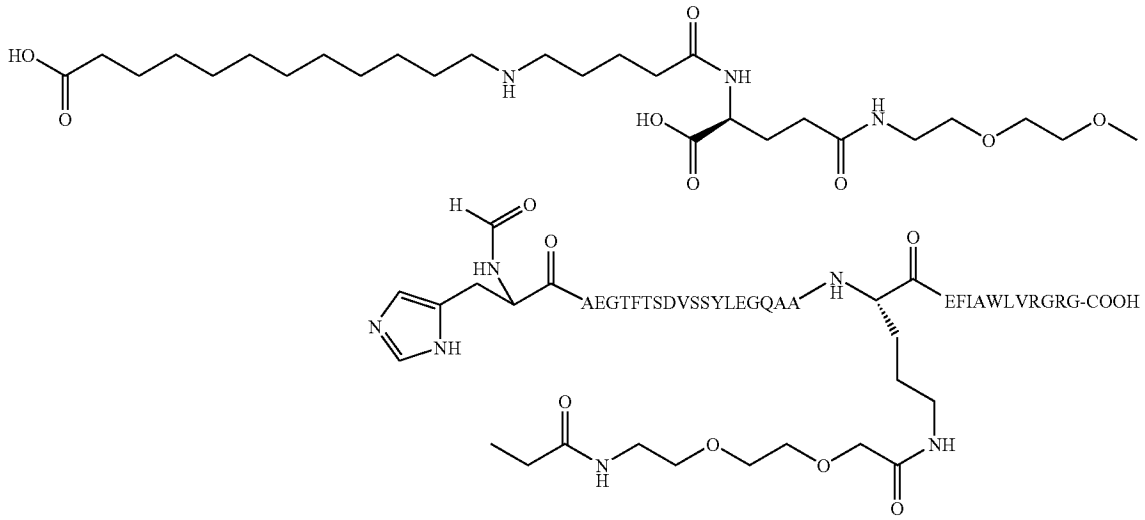
N-ϵ^{26}-[2-(2-[2-(2-[2-(2-[4-{[N-(2-carboxyethyl)-N-(17-carboxyheptadecanoyl)amino]methyl}benzoyl)amino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1(7-37)

[Aib8,Arg34]GLP-1(7-37) peptide starting from 150 mg 2-chlorotrityl chloride resin (1.4 mmol/g) was prepared by Fmoc-solid phase peptide synthesis using Apex396 from Advanced Chemtech. The Lys residue at position 26 was protected as Lys(ivDde) while the functional side-chains for the other amino acids were protected with standard acid labile protecting groups. The Lys residue was deprotected with 3% hydrazine/3% piperidine in NMP for 1 hr. The two units of 8-amino-3,6-dioxaoctanoic acid and 4{[(2-tert-butoxycarbonyl-ethyl)-(17-tert-butoxycarbonyl-heptadecanoyl)-amino]-methyl}-benzoic acid were coupled to the resin attached peptide using DIC/HOAt. The peptide was finally deprotected and cleaved from the resin with TFA/TIS/H_2O/thioanisol (90/5/3/2). The peptide was isolated by preparative LC-MS.

HPLC: Elutes at 52% acetonitrile

MALDI: 4191 (MH+)

Example 14

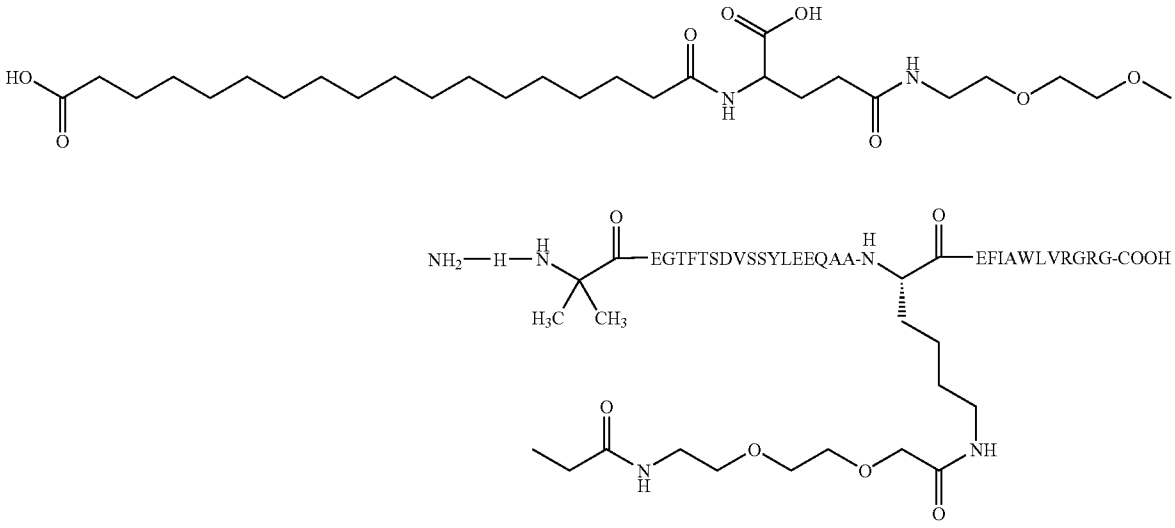


N-α^7-formyl,N-ϵ^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Arg34]GLP-1-(7-37)-peptide

HPLC (method B6): RT=32.6 min

LCMS: m/z=1377.3 (MH_3^{3+}) Calculated for (M^+)=4128.0

Example 15



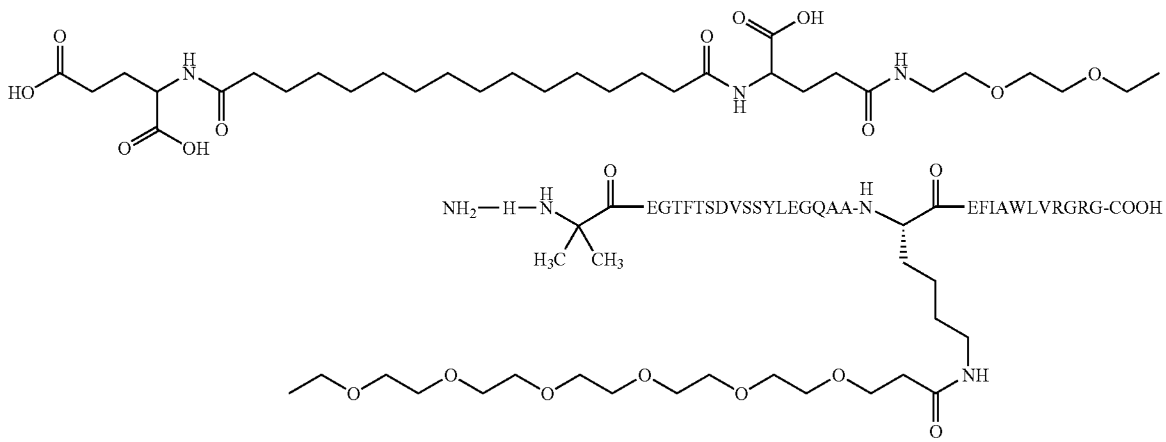
N-ϵ[26]26-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Glu22,Arg34]GLP-1-(7-37)peptide

[Aib8,Glu22,Arg34]GLP-1(7-37) peptide starting from 150 mg Fmoc-Gly-Wang resin (0.66 mmol/g) was prepared by Fmoc-solid phase peptide synthesis using Apex396 from Advanced Chemtech. The Lys residue at position 26 was protected as Lys(Mtt) while the functional side-chains for the other amino acids were protected with standard acid labile protecting groups. The Lys residue was deprotected with 2% TFA/2% TIS in DCM for 4×5 min. The two units of 8-amino-3,6-dioxaoctanoic acid, γ-glutamic and octadecanoic acid tert-butyl ester were coupled to the resin attached peptide using DIC/HOAt. The peptide was finally deprotected and cleaved from the resin with TFA/TIS/H_2O/thioanisol (90/5/3/2). The peptide was isolated by LC-MS.

HPLC: Elutes at 50% acetonitrile

MALDI: 4187 (MH+)

Example 16



N-ϵ[26]{3-[2-(2-{2-[2-(2-{2-[2-(2-[4-(15-(N—((S)-1,3-dicarboxypropyl)carbamoyl)pentadecanoylamino)-(S)-4-carboxybutyrylamino]ethoxy)ethoxy]ethoxy}ethoxy)ethoxy]ethoxy}ethoxy)ethoxy]propionyl}[Aib8,Arg34]GLP-1-(7-37)-peptide

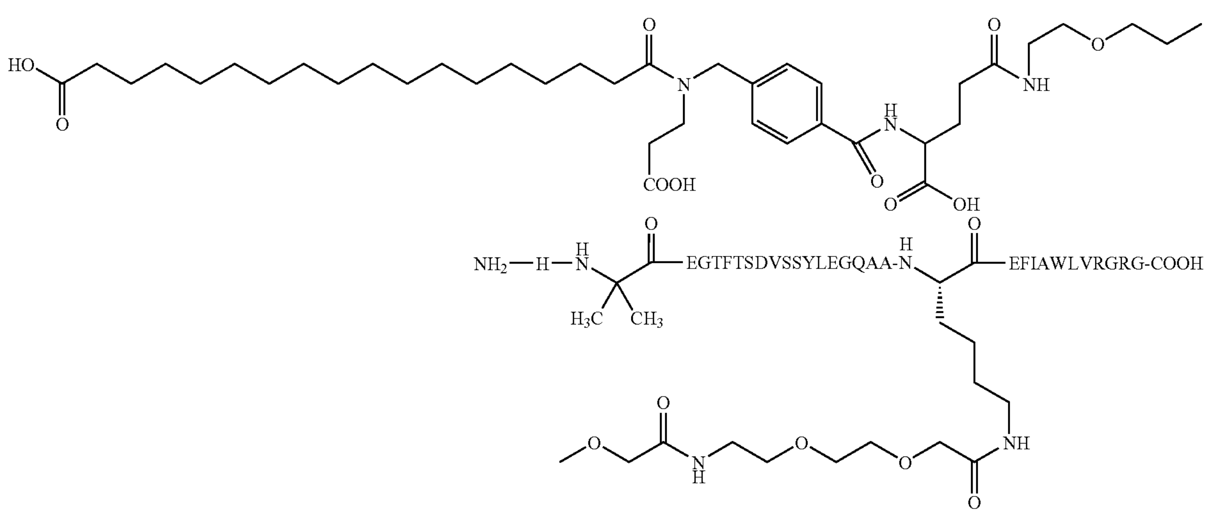
Method and Analysis

Prepared as in Example 3 and in accordance with "synthetic methods".

HPLC (method B4): RT=10.29 min (92%)

LCMS: m/z=1450 (MH_3^{3+}). Calculated for (MH_3^{3+}): 1450

Example 17



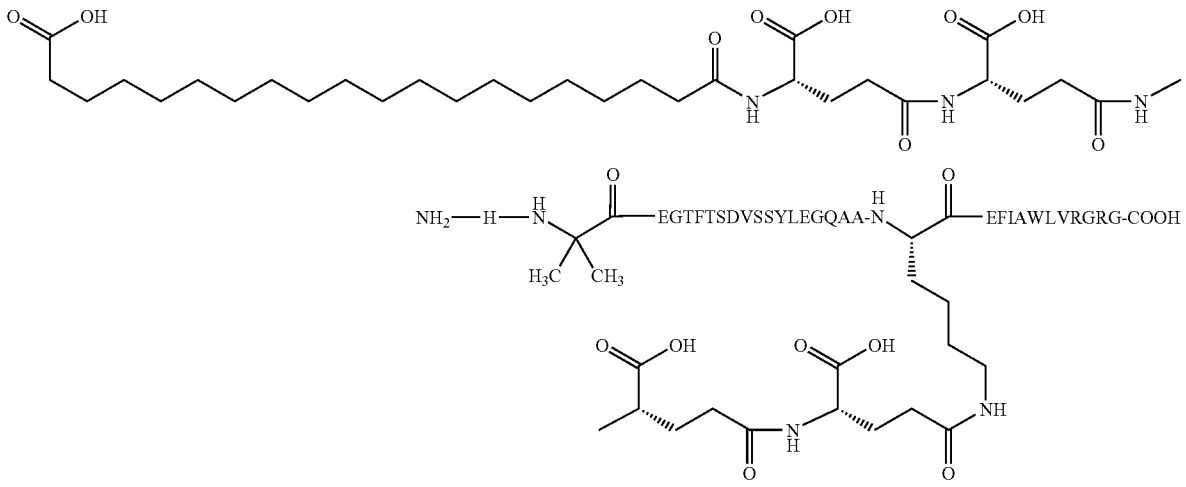
N-ϵ^{26}-[2-(2-[2-(2-[2-(2-[4-{[N-(2-carboxyethyl)-N-(17-carboxyheptadecanoyl)amino]methyl}benzoyl)amino](4(S)-carboxybutyrylamino)ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1(7-37)

[Aib8,Arg34]GLP-1(7-37) peptide starting from 150 mg Fmoc-Gly Wang resin (0.66 mmol/g) was prepared by Fmoc-solid phase peptide synthesis using Apex396 from Advanced Chemtech. The Lys residue at position 26 was protected as Lys(Mtt) while the functional side-chains for the other amino acids were protected with standard acid labile protecting groups. The Lys residue was deprotected with 2% TFA/2% TIS in DCM for 4×5 min. The two units of 8-amino-3,6-dioxaoctanoic acid, γ-glutamic acid and 4{[(2-tert-butoxycarbonyl-ethyl)-(17-tert-butoxycarbonyl-heptadecanoyl)-amino]-methyl}-benzoic acid were coupled to the resin attached peptide using DIC/HOAt. The peptide was finally deprotected and cleaved from the resin with TFA/TIS/H_2O/thioanisol (90/5/3/2). The peptide was isolated by preparative HPLC.

HPLC: Elutes at 51% acetonitrile

MALDI: 4320 (MH+)

Example 18



Nε^{26}-{(S)-4-carboxy-4-((S)-4-carboxy-4-((S)-4-carboxy-4-((S)-4-carboxy-4-(19-carboxynonadecanoylamino)butyrylamino)butyrylamino)butyrylamino)butyrylamino}[Aib8,Arg 34]GLP-1-(7-37)

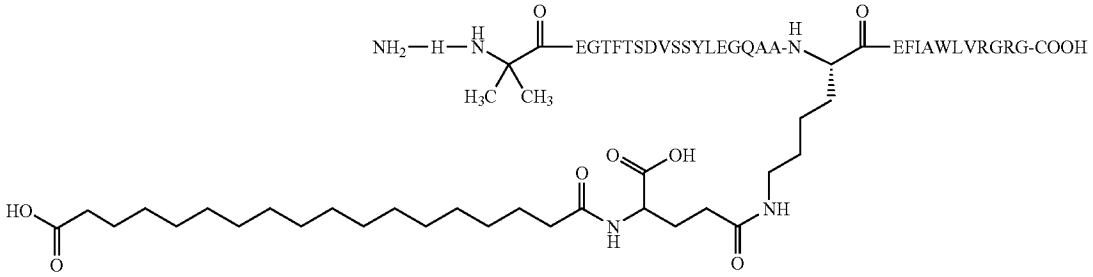
The peptide was synthesized using Fmoc chemistry on a Liberty Microwave Peptide Synthesizer (CEM Corporation). The synthesis was performed on a Gly-Wang resin (Novabiochem) with a loading of 0.66 mmol/g using 4 fold excess of amino acids and DIC/HOAt for coupling. The N-terminal histidine was Boc-protected and the lysine to be modified was Mtt-protected. After synthesis of the peptide backbone, the Mtt group was removed with 3% TFA in DCM and the side chain was built on the Liberty using standard peptide synthesis protocols. In the last step the fatty diacid was added as a mono-t-butyl-ester.

After cleavage with TFA/TIS/water (95:2.5:2.5), the peptide was dissolved in 50% acetonitrile by addition of DIPEA and purified on a Waters LC-MS system using a 7.8×300 mm X-Terra Prep MS C18 10 μm column running at room temperature. After 5 minutes at 30% CH_3CN, 0.08% TFA, 4 ml/min, the column was eluted with a linear gradient of 30 to 70% CH_3CN over 40 minutes. The fractions containing the desired compound were collected and the concentration of the peptide in the eluate was determined by measurement of the UV absorption at 280 nm assuming molar extinction coefficients of 1280 and 3690 for tyrosine and tryptophan respectively. The identity and purity was confirmed by MALDI. After the concentration determination the eluate was aliquotted into vials containing the desired amount and dried by vacuum centrifugation.

HPLC: Elutes at 52% acetonitrile

MALDI: 4239 (MH+)

Example 19



N-ε^{26}-4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyryl-[Aib8,Arg34]GLP-1-(7-37)-peptide

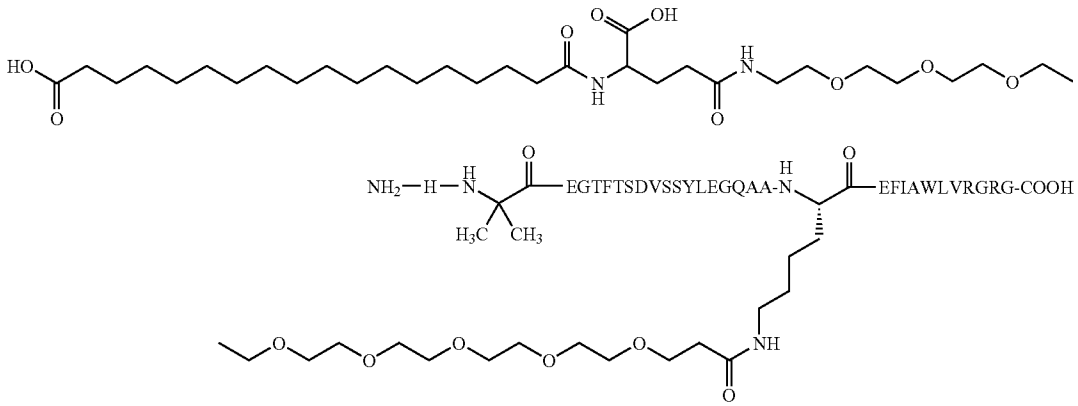
Method and Analysis

Prepared as in Example 4 and in accordance with "synthetic methods".

HPLC (method B4): Rt=9.64 min (97%)

LCMS: m/z:=1276 (MH_3^{3+}), Calculated for (MH_3^{3+}) 1276

Example 20

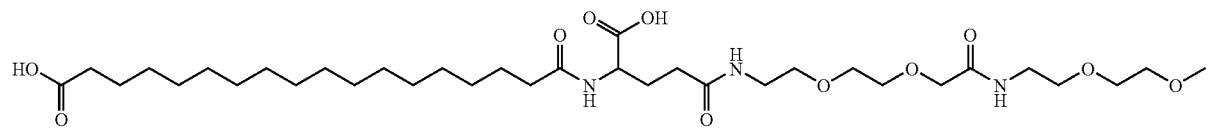


N-ε^{26}-{3-[2-(2-{2-[2-(2-{2-[2-(2-[4-(17-carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]ethoxy}ethoxy)ethoxy]ethoxy}ethoxy)ethoxy]propionyl}[Aib8,Arg34]GLP-1-(7-37)-peptide

LCMS*: m/z:=1417 (MH_3^{3+}), Calculated for (MH_3^{3+}) 1417

*HPLC (Eluted at 0.5 mL/min at 42° C. by a linear gradient from 5---->80% acetonitrile, 85---->10% water and 10% of a solution of 1.0% trifluoroacetic acid over 50 min. UV detection at 214 on a Symmetry300, 5 um, 3.9 mm×150 mm C-18 silica column.)method B4): Rt=32.09 min (95%)

Example 21



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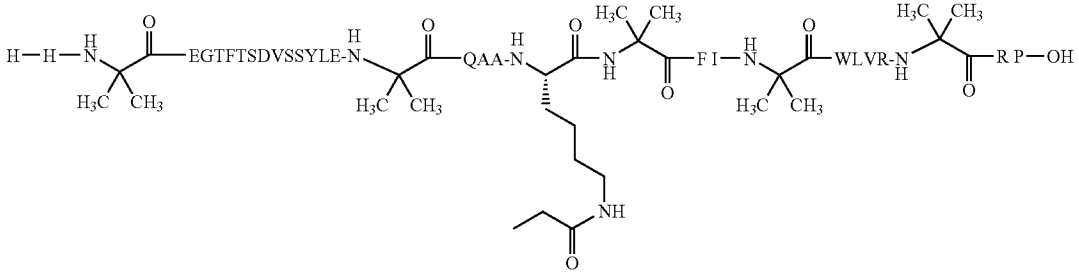
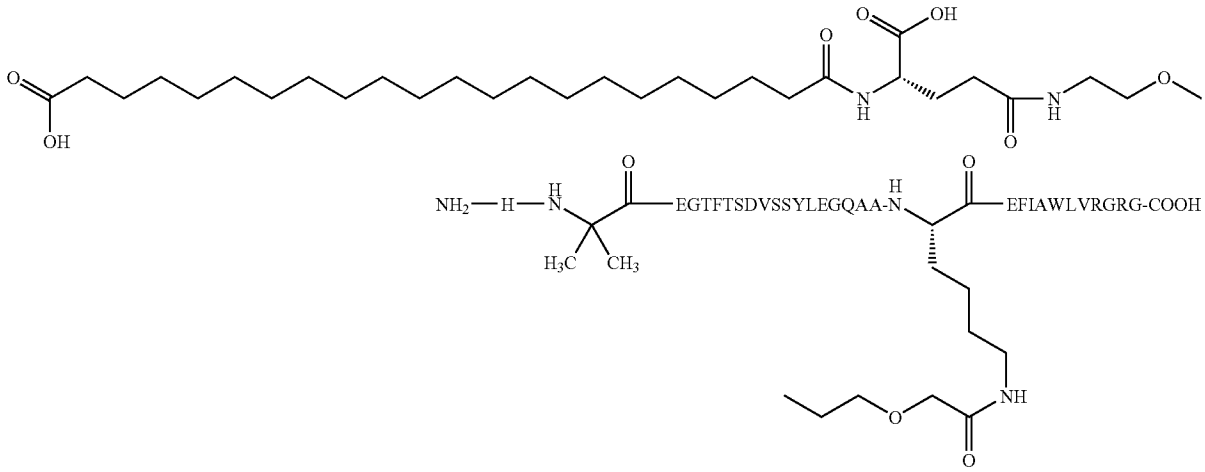


N-ε^{26}-{2-(2-(2-(2-[2-(2-(4-(17-carboxyheptadecanoylamino)-4-carboxybutyrylamino)ethoxy)ethoxy]acetyl)ethoxy)ethoxy)acetyl)}-[Aib^{8,22,27,30,35}, Arg^{34},Pro^{37},Lys^{26}]GLP-1(7-37) amide

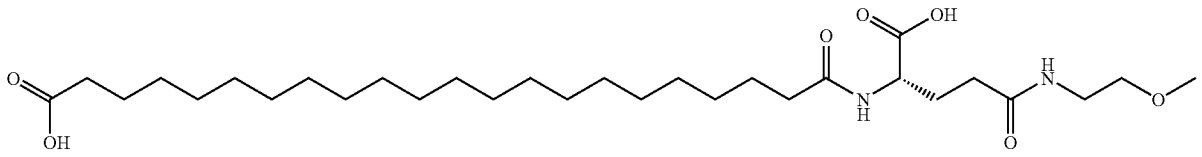
HPLC (method B6): RT=35.0 min
LCMS: m/z=1394.0 (MH_3^{3+}) Calculated for (M^+)=4180.0

Example 22



N-ε^{26}-[2-(2-[2-[4-(21-Carboxyuneicosanoylamino)-4(S)-carboxybutyrylamino]ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)

Prepared using the same method as in Example 19
HPLC: Elutes at 53.4% acetonitrile
MALDI: 4025 (MH+)
Other compounds of this invention include:



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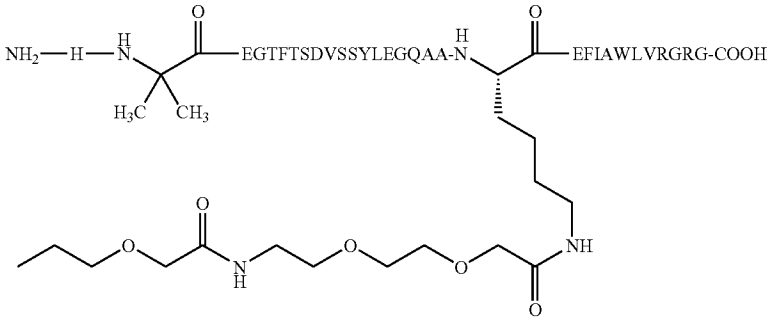

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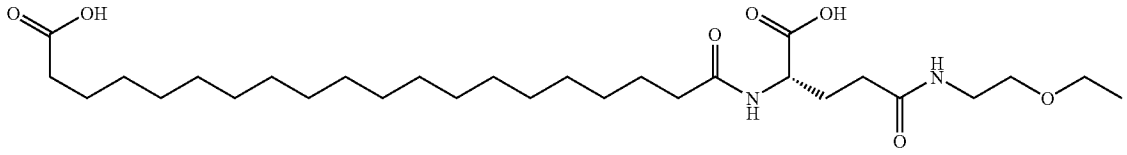
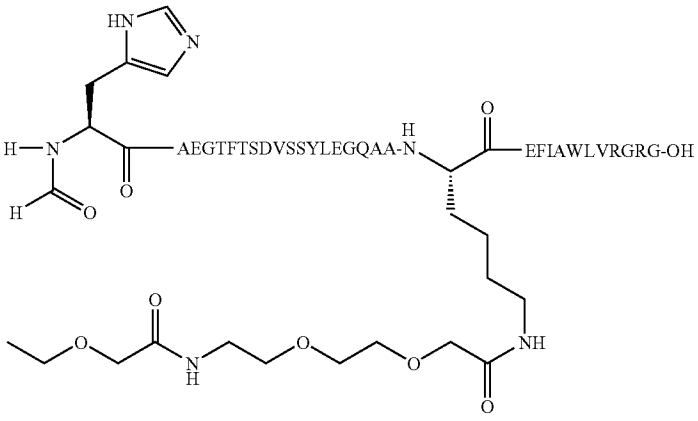
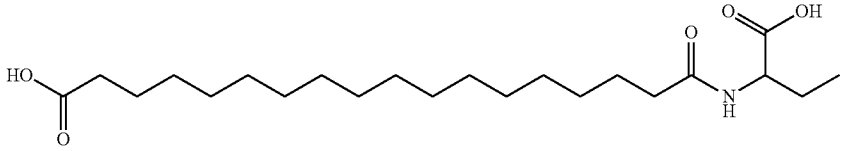
N-ϵ^{26}-[2-(2-[2-(2-[2-(2-[4-(21-Carboxyuneicosanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide





N-α1-formyl-N-ϵ^{26}-[2-(2-[2-(2-[2-(2-[4-(19-Carboxynonadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Arg34]GLP-1-(7-37)peptide



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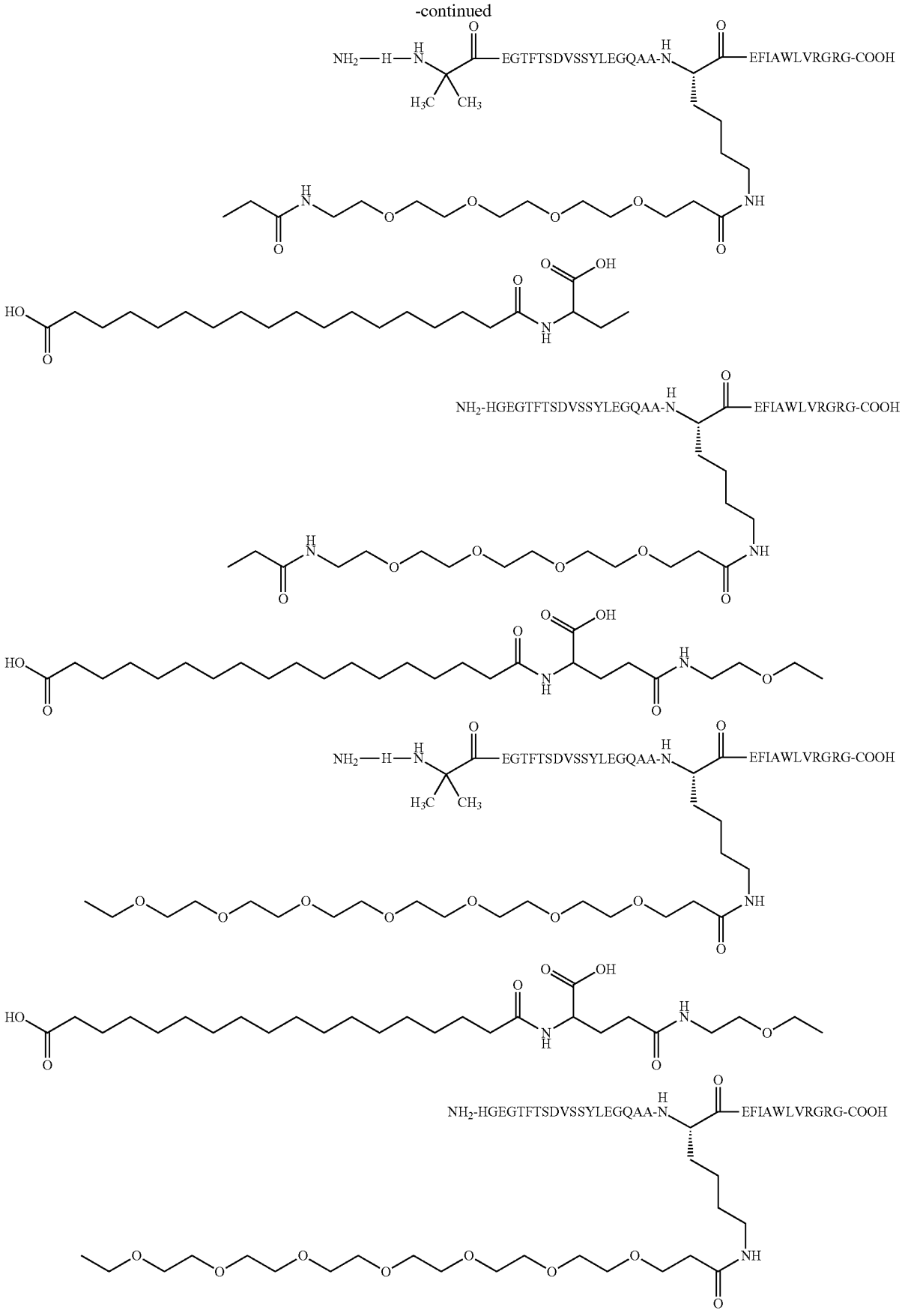
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OH
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EFIAWLVRGRG-COOH
NH2—H—N
EGTFTSDVSSYLEGQAA-N
EFIAWLVRGRG-COOH
H3C CH3
HO
OH
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EFIAWLVRGRG-COOH

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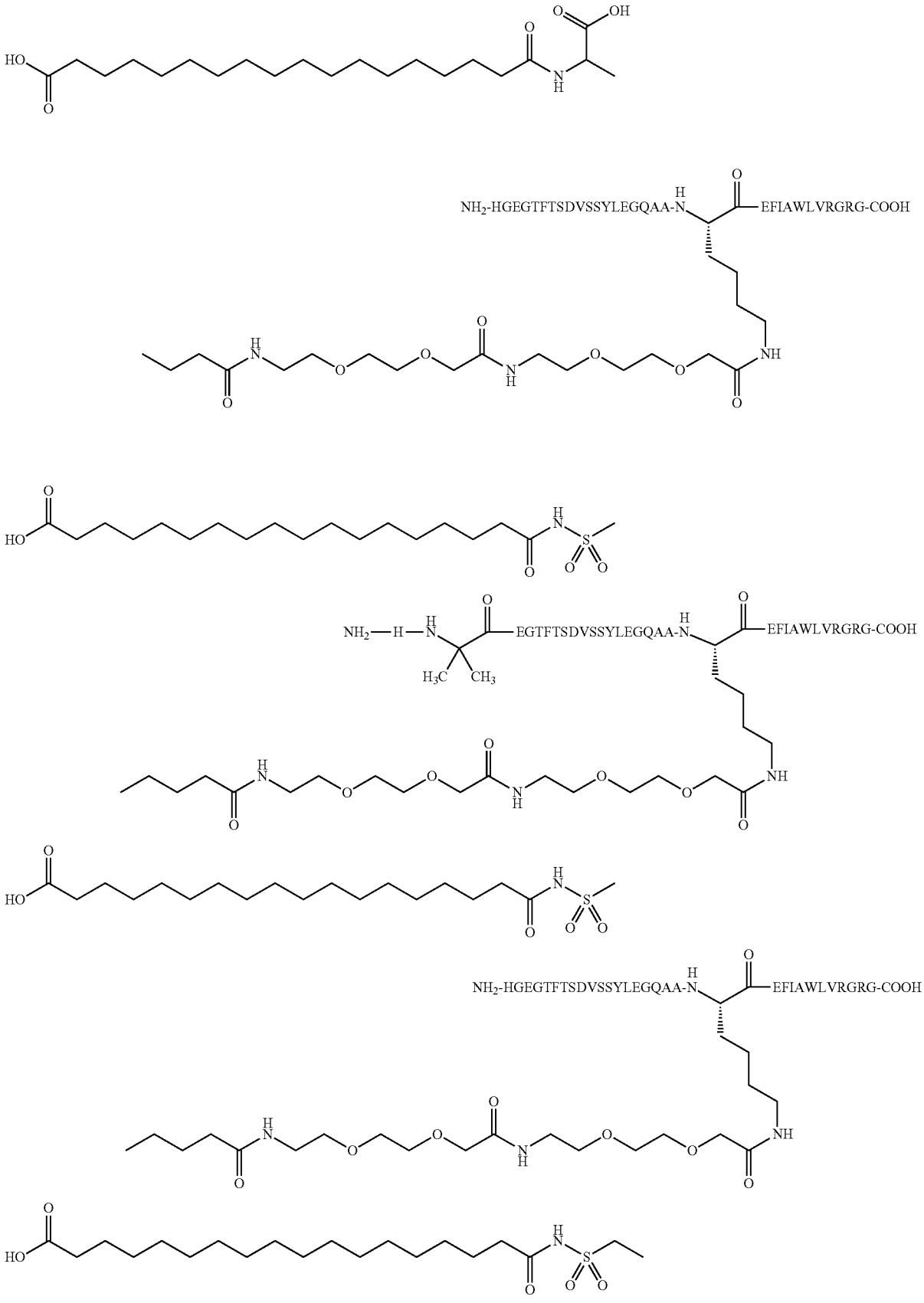
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NH
S
NH2
H3C
CH3

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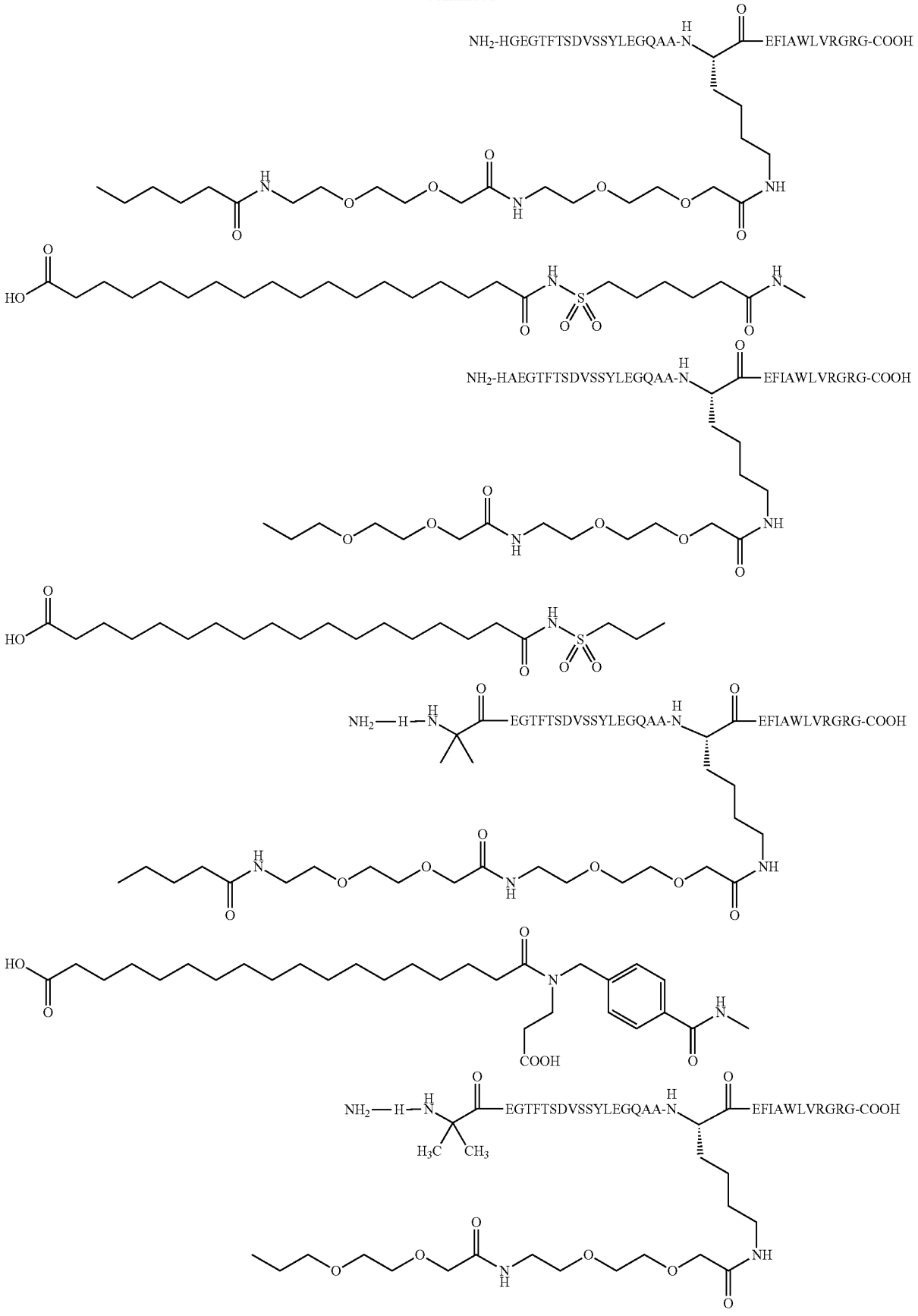
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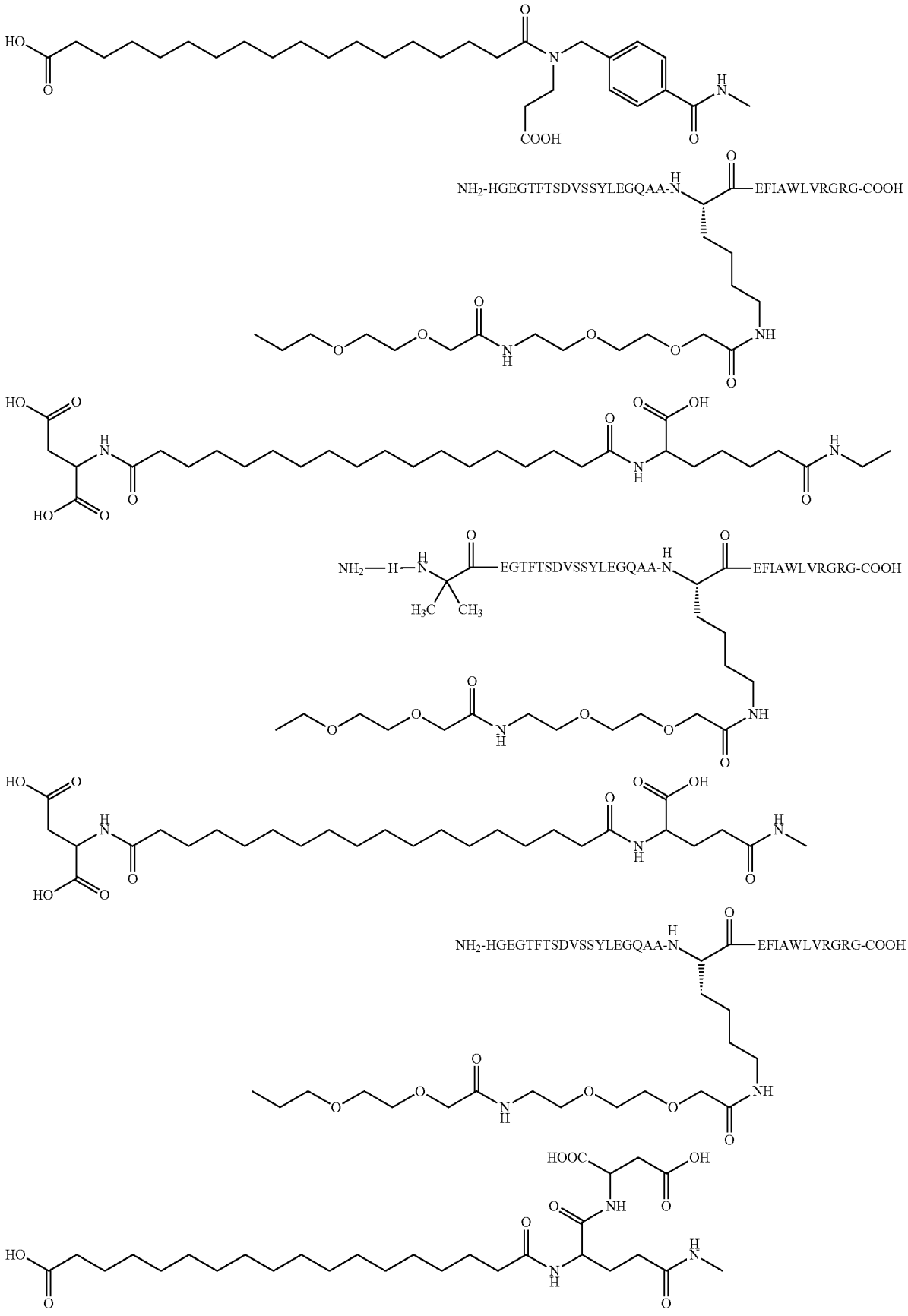
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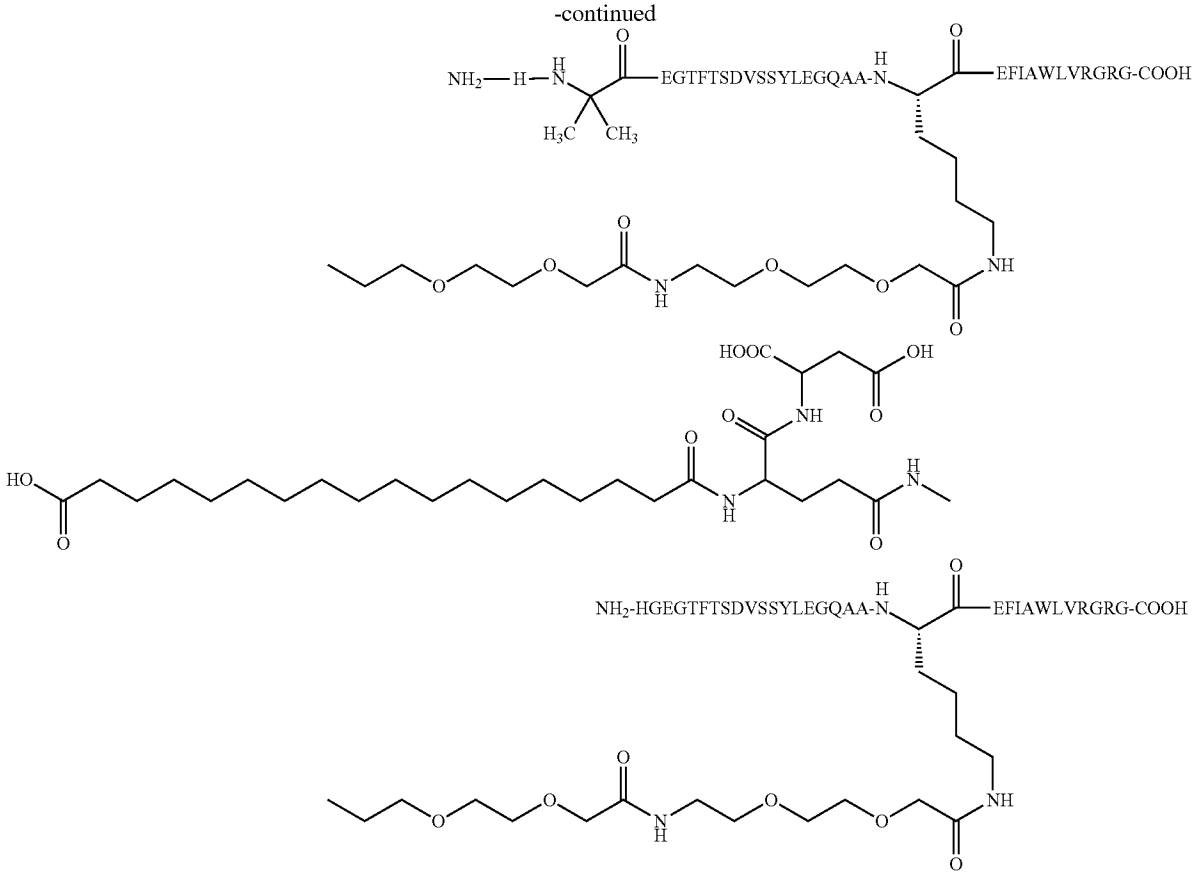

Pharmacodynamic Study Using db/db Mice

In one aspect of this invention the GLP-1 agonists have a duration of action of at least 24 hrs after dosing of 30 nmol/kg to db/db mice

The efficacy and duration of action are measured in db/db mice.

Male db/db mice are shipped from Taconic, Denmark at the age of 8-10 weeks. From the time of arrival, the mice are housed under standard conditions but at 24° C. The mice are kept 10 per cage until experimentation with free access to standard chow (Altromin, Brogaarden APS., Denmark) and tap water at a normal day: light cycle (light on at 6 am). The mice are used for 1 experiment per week for 3 weeks. After this, the mice are euthanized.

After an acclimatisation period of 1 week, the blood glucose is measured by sampling from the tail tip capillary. In brief, 5 μl blood is sampled in heparinised glass capillary tubes and immediately suspended in 250 μl EBIO buffer solution (Eppendorf, Germany) in an 1.5 ml Eppendorf tube. The blood glucose concentration is measured by the glucose oxidase method at the EBIO Plus Auto analyser (Eppendorf, Germany).

The cut of value for blood glucose is 10 mM. When evaluating the mice, it is essential, that all 42 mice entering the experiment have blood glucose values above 10 mM, but also that the inter-mice variance is small. Therefore, if many mice are not severely diabetic, whereas some are, the start up of experiments should be postponed one week and new basal blood glucose measurements be made.

Based on the basal blood glucose values, the mice are allocated to 7 groups of n=6 with matching group mean blood glucose values.

On the day of testing the basal blood glucose morning values are assessed as described above and the basal body weight of each mouse is assessed. A time 0, the compound is dosed subcutaneously in the scruff of the neck (dosing volume app. 300 μl/50 g mouse).

The blood glucose values are followed up to 48 hours (time 1, 3, 6, 24 and 48 h) and the terminal body weight is assessed.

All data are entered into Graphpad Prism where mean blood glucose and mean delta body weights are calculated.

One aspect of this invention is to prepare GLP-1 analogues/derivatives with extended plasma half-lives that are suitable for once weekly administration. The pharmaco kinetic properties can be evaluated in mini pigs or domestic pigs as described below

Pharmacokinetic Screening of Once Weekly GLP-1 Analogues

Pharmacokinetic screening of GLP-1 analogues for identification of suitable once weekly candidates were performed on candidates that according to the project screenings plan were shown to be sufficiently potent with respect to glucose lowering potential in a diabetic mouse model (db/db mice) and subsequently had a time of duration of 48 hours or more in the db/db mouse model.

Primary Screening

The first part of the pharmacokinetic screening consisted of a single dose subcutaneous administration of 2 nmol/kg to three minipigs weighing 8-12 kg. Blood samples were drawn from each animal at predose, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96 and 120 hours post-injection. All blood samples were stabilised with a special stabilisation buffer consisting of:

EDTA (di-sodium) 0.18 M, Aprotenin 15000 KIE/ml, Val-Pyr 0.30 mM, pH adjusted to 7.4 in order to prevent enzy-

matic degradation of the GLP-1 analogues. Plasma was collected from each stabilised blood samples by centrifugation (4° C., 10 min., 1270 G (4000 rpm), and analysed for the content of GLP-1 analogue by ELISA assays. Three different ELISA assays were used for the plasma analysis: "The "Total assay" using the antibody combination F1/Ra2135 detecting both the N-terminally intact 7-37GLP-1 molecule and the N-terminal enzymatically degraded 9-37GLP-1 molecule with a limit of detection (LOD) of 35 pM and a dynamic analytical range of 35-30000 pM. The "Intact assay" using the antibody combination F1/Mab26.1. This assay was detecting the N-terminally intact 7-37GLP-1 molecule only. The LOD was 35 pM and a dynamic analytical range of 35-30000 pM. The "Aib-intact assay" using the antibody combination F1/GLP162-3F15. This assay was detecting the Aib stabilised N-terminal of the GLP-1 molecule enabling detection of stabilised GLP-1 analogues. The LOD was 45 pM and the dynamic analytical range 45-30000 pM.

All plasma concentration-time profiles were analysed pharmacokinetically by a non-compartmental analysis. The following pharmacokinetic parameters were calculated if data permitted: t_{max}, C_{max}, AUC, AUC/Dose, $AUC_{\%Extrapol}$, λ_z, $t_{1/2}$, CL/F, V_z/F and MRT.

Secondary Screening

A second part of the pharmacokinetic screening was conducted on those compounds with an initial terminal half-life of 60-70 hours or more. This screening consisted of a single dose intravenous and subcutaneous administration of 2 nmol/kg to six minipigs for each route of administration. The blood sampling schedule was extended from 0-120 hours to 0-432 and 0-504 hours after intravenous and subcutaneous administration respectively. This was done in order to increase the precision and accuracy of the pharmacokinetic parameter estimates, especially the terminal half-life, AUC and the derived parameters clearance and volume of distribution, and to estimate the bioavailability after subcutaneous administration.

GLP-1 (AIB8-Intact) Assay

The assay was a two-site assay with simultaneous incubation of the analyte with catcher and detector antibody. A ready to use chemiluminescent substrate was used to maximize signal. The assay neither recognizes endogen GLP-1(7-37) nor the DPPIV cleaved GLP-1(9-37).

Reference Plasma for GLP-1 Assays

0-plasma was prepared from pooled EDTA plasma without Valine Pyrrolidide and Aprotinin from fasting animals. The pooled EDTA plasma was incubated at 37° C. for 4 hours to remove traces of GLP-1 and after incubation Valine Pyrrolidide and Aprotinin were added.

Buffers

Coating Buffer

PBS was used as coating buffer: 10 mM sodium phosphate and 145 mM sodium chloride adjusted to pH 7.4.

Washing Buffer

PBS with 0.05% (v/v) Tween 20

Assay Buffer

PBS with 0.05% (v/v) Tween 20.10 g/L BSA and 10 mg/L anti-TNP.

Streptavidin Buffer

Washing buffer with an additional 0.5M NaCl.

Substrate

Ready to use substrate SuperSignal ELISA Femto (Pierce, cat. no. 37075).

Standards

Standards were prepared from a 25 μM stock solution of 0113-0000-0217. The peptide was serially diluted into reference plasma to make standards with final concentrations of 30000-10000-3333-1111-370-123-41 and 0 pM. Standards were stored in Micronic tubes in 100 μL aliquots at −20° C.

Assay Procedure

Crystal 2000 Microplates (black) were coated with monoclonal antibody GLPb1-7F1, 100 μL of 5 μg/mL in PBS overnight at 4° C.

Plates were washed 5 times with washing buffer in an automated plate washer (SkanWasher, Skatron) and allowed to stand for at least 30 min. with washing buffer to block remaining sites.

20 μL of sample or standard was added to each well in duplicate immediately followed by 100 μL GLP162-3F15 biotinylated, 1 μg/mL in assay buffer. Plates were incubated for 2 hours at room temperature on a plate shaker followed by 5 wash cycles as previously described. 100 μL of streptavidin-peroxidase solution (KPL, code 14-30-00, 1:20000 in streptavidin buffer) was added to each well and incubated for 1 hour at room temperature on a plate shaker. Plates were washed as previously described and after emptying 100 μL of SuperSignal femto was added. Plates were put on a shaker for 1 minute and measured in Orion Luminometer (Berthold). Data were transferred to MultiCalc and standard curves calculated using the weighted 4PL method. Sample concentrations were calculated from the standard curve.

GLP-1 (Total) Assay

The assay was a two-site assay with simultaneous incubation of the analyte with catcher and detector antibody. The assay recognizes N-terminally cleaved GLP-1 up to GLP-1 (12-37).

Buffers

Coating Buffer

PBS was used as coating buffer: 10 mM sodium phosphate and 145 mM sodium chloride adjusted to pH 7.4.

Washing Buffer

PBS with 0.05% (v/v) Tween 20

Assay Buffer

PBS with 0.05% (v/v) Tween 20.10 g/L BSA and 10 mg/L anti-TNP.

Streptavidin Buffer

Washing buffer with an additional 0.5M NaCl.

Substrate

Ready-to-use substrate TMB (KemEnTec code 4380A)

Stop Buffer

4 M H_3PO_4

Standards

Standards were prepared from a 25 μM stock solution of 0113-0000-0217. The peptide was serially diluted into reference plasma to make standards with final concentrations of 30000-10000-3333-1111-370-123-41 and 0 pM. Standards were stored in Micronic tubes in 100 μL aliquots at −20° C.

Assay Procedure

Maxisorp microtiter plates (NUNC) were coated with monoclonal antibody GLPb1-7F1, 100 μL of 5 μg/mL in PBS overnight at 4° C.

Plates were washed 5 times with washing buffer in an automated plate washer (SkanWasher, Skatron) and allowed to stand for at least 30 min. with washing buffer to block remaining sites.

20 μL of sample or standard was added to each well immediately followed by 100 μL Ra2135-biotinylated, 1 μg/mL in assay buffer. Plates were incubated for 2 hours at room temperature on a plate shaker followed by 5 wash cycles as previously described.

100 μL of streptavidin-peroxidase solution (Amersham Biosciences code RPN4401V, 1:8000 in assay buffer) was added to each well and incubated for 1 hour at room temperature on a plate shaker. Plates were washed as previously

described and after emptying 100 μL of TMB was added and after 5 minutes stopped with 100 μL H_3PO_4.

Plates were measured in Victor Multilabel Reader (Wallac). Data were transferred to MultiCalc and standard curves calculated using the weighted 4PL method. Sample concentrations were calculated from the standard curve.

The in-life experimental procedures, plasma analysis and pharmacokinetic analysis were identical to that described under the primary screening.

Pharmaceutical Formulation:

A compound of the invention may be formulated as:

Compound of example 4	6.25 mg/ml
Propyleneglycol	14.0 mg/ml
Phenol	5.5 mg/ml
Phosphate Buffer	pH 8.15

Optionally the compound is treated with heat and/or base before formulation as described in PCT/EP2005/055946.

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Xaa Xaa Glu Gly Thr Phe Thr Ser Asp Xaa Ser Xaa Xaa Xaa Glu Xaa
1 5 10 15

Xaa Ala Xaa Lys Xaa Phe Ile Xaa Trp Leu Xaa Xaa Xaa Xaa Xaa Xaa
20 25 30

<210> SEQ ID NO 4
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 4

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His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1               5                   10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
            20                  25                  30


<210> SEQ ID NO 5
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 5

His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1               5                   10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
            20                  25                  30


<210> SEQ ID NO 6
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 6

His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1               5                   10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
            20                  25                  30


<210> SEQ ID NO 7
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 7

His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1               5                   10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
            20                  25                  30


<210> SEQ ID NO 8
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
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<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 8

His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 9
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 9

His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 10
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 10

His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 11
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 11

His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

-continued

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 12
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 12

His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 13
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 13

His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 14
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 14

His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 15
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 15

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His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1               5                   10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
            20                  25                  30


<210> SEQ ID NO 16
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 16

His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1               5                   10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
            20                  25                  30


<210> SEQ ID NO 17
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 17

His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1               5                   10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
            20                  25                  30


<210> SEQ ID NO 18
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 18

His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1               5                   10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
            20                  25                  30


<210> SEQ ID NO 19
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
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<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 19

His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 20
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 20

His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 21
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 21

His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 22
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 22

His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 23
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 23

His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 24
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 24

His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 25
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa = 3-(4-imidazolyl)propionyl
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 25

Xaa Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 26
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: VARIANT

<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 26

His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 27
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 27

His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 28
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 28

His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 29
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 29

His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Pro
20 25 30

<210> SEQ ID NO 30
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 30

His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 31
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa = N-alpha-formyl-histidine
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 31

Xaa Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 32
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 32

His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 33
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 33

His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 34
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 34

His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 35
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 35

His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 36
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic
<220> FEATURE:
<221> NAME/KEY: VARIANT

<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 36

His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 37
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 37

His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

<210> SEQ ID NO 38
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (16)..(16)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (24)..(24)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (29)..(29)
<223> OTHER INFORMATION: Xaa = Aib

<400> SEQUENCE: 38

His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Xaa
1 5 10 15

Gln Ala Ala Lys Xaa Phe Ile Xaa Trp Leu Val Arg Xaa Arg Pro
20 25 30

<210> SEQ ID NO 39
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 39

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His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1               5                   10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
            20                  25                  30
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<210> SEQ ID NO 40
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Aib
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 40

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His Xaa Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1               5                   10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
            20                  25                  30
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<210> SEQ ID NO 41
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa = N-alpha-formyl-histidine
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Lys is substituted

<400> SEQUENCE: 41

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Xaa Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1               5                   10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
            20                  25                  30
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<210> SEQ ID NO 42
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic
<220> FEATURE:
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Xaa Ala Xaa Lys Xaa Phe Ile Xaa Trp Leu Xaa Xaa Xaa Xaa Xaa
20 25 30

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<400> SEQUENCE: 43

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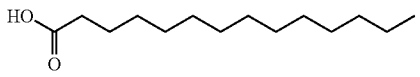
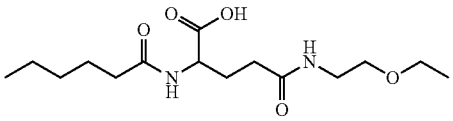
1 5 10 15

Xaa Ala Xaa Lys Xaa Phe Ile Xaa Trp Leu Xaa Xaa Xaa Xaa Xaa
20 25 30

The invention claimed is:

1. A compound of the structure





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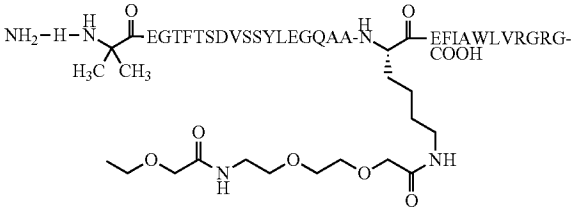
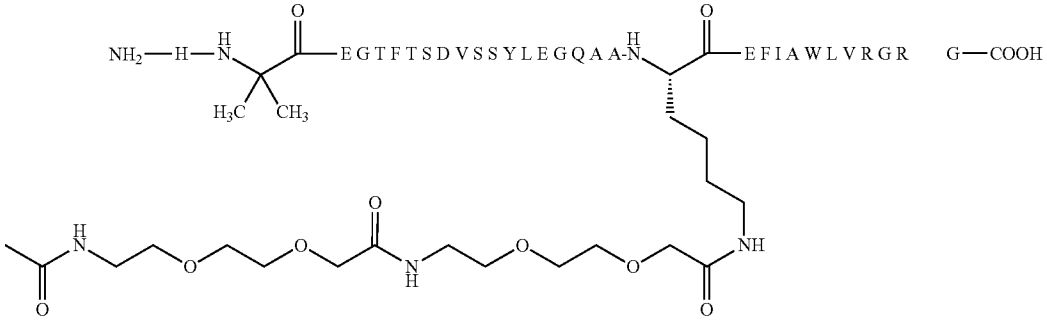
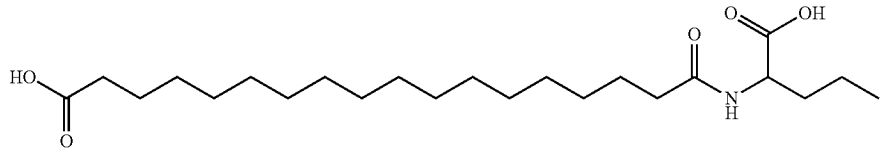


where the amino acid sequence is that of SEQ ID NO: 7.

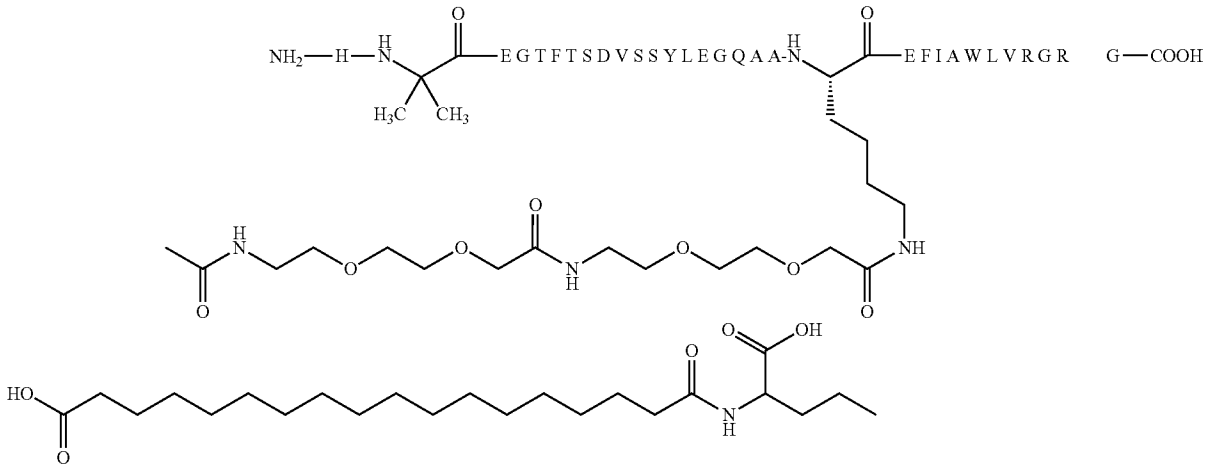
2. A pharmaceutical composition comprising a compound of the structure





where the amino acid sequence is that of SEQ ID NO: 7, and a pharmaceutical excipient.

3. A method for treating type 2 diabetes in a subject, said method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising a compound of the structure



where the amino acid sequence is that of SEQ ID NO: 7, and a pharmaceutically acceptable excipient.

4. A compound having the following name N-ϵ^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide.

5. A pharmaceutical composition comprising a compound having the following name N-ϵ^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide and a pharmaceutically acceptable excipient.

6. A method for treating type 2 diabetes in a subject, said method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising a compound having the following name N-ϵ^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide and a pharmaceutically acceptable excipient.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 8,129,343 B2
APPLICATION NO. : 11/908834
DATED : March 6, 2012
INVENTOR(S) : Lau et al.

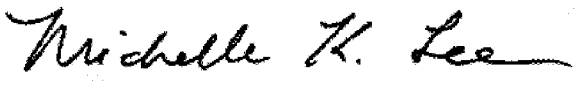
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the Title Page:

The first or sole Notice should read --

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 1114 days.

Signed and Sealed this
First Day of September, 2015



Michelle K. Lee
Director of the United States Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

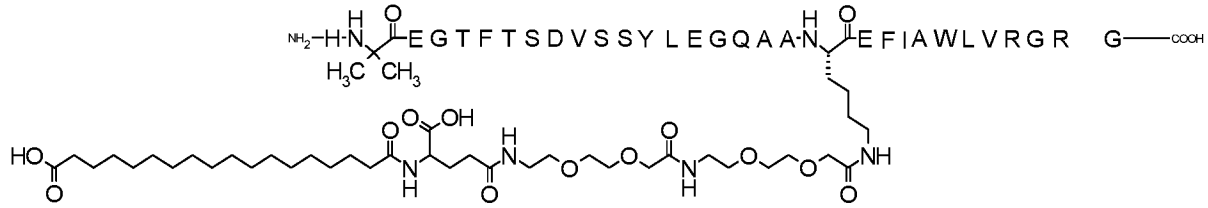
PATENT NO. : 8,129,343 B2
APPLICATION NO. : 11/908834
DATED : March 6, 2012
INVENTOR(S) : Lau et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Claims

In column 129, claim 1, please allow the claim to read as follows:

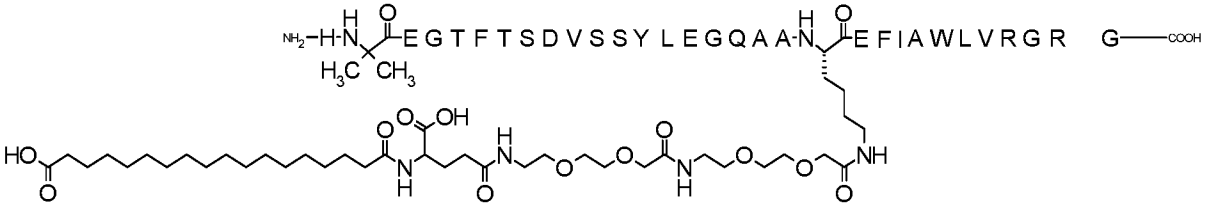
1. A compound of the structure



where the amino acid sequence is that of SEQ ID NO:7.

In column 130, claim 2, please allow the claim to read as follows:

2. A pharmaceutical composition comprising a compound of the structure



where the amino acid sequence is that of SEQ ID NO:7, and a pharmaceutically acceptable excipient.

Signed and Sealed this
Twenty-ninth Day of March, 2016

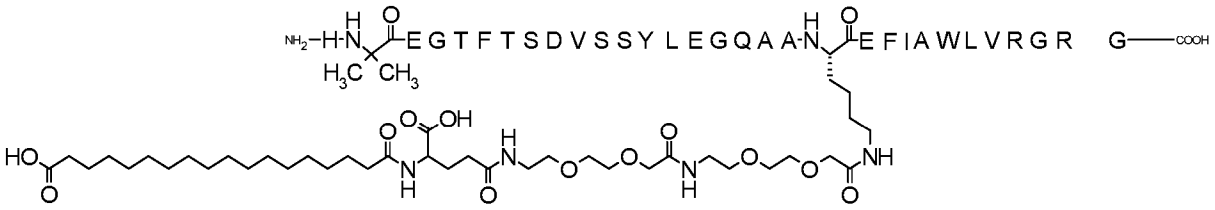
Michelle K. Lee

Michelle K. Lee
Director of the United States Patent and Trademark Office

CERTIFICATE OF CORRECTION (continued)
U.S. Pat. No. 8,129,343 B2

In column 131, claim 3, please allow the claim to read as follows:

3. A method for treating type 2 diabetes in a subject, said method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising a compound of the structure



where the amino acid sequence is that of SEQ ID NO:7, and a pharmaceutically acceptable excipient.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO.	: 8,129,343 B2
APPLICATION NO.	: 11/908834
DATED	: March 6, 2012
INVENTOR(S)	: Jesper Lau et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the Title Page:

The first or sole Notice should read --

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 1046 days.

Signed and Sealed this
Seventeenth Day of October, 2017

Joseph Matal
Performing the Functions and Duties of the
Under Secretary of Commerce for Intellectual Property and
Director of the United States Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE

(12) CERTIFICATE EXTENDING PATENT TERM
UNDER 35 U.S.C. § 156

(68)	PATENT NO.	:	8,129,343
(45)	ISSUED	:	March 6, 2012
(75)	INVENTOR	:	Lau et al.
(73)	PATENT OWNER	:	NoVo Nordisk A/S
(95)	PRODUCT	:	OZEMPIC® (semaglutide)

This is to certify that an application under 35 U.S.C. § 156 has been filed in the United States Patent and Trademark Office, requesting extension of the term of U.S. Patent No. 8,129,343 based upon the regulatory review of the product OZEMPIC® (semaglutide) by the Food and Drug Administration. According to United States Patent and Trademark Office records, the original expiration date of the patent as of the date of issuance of this certificate is January 29, 2029. Because it appears that the requirements of the law have been met, this certificate extends the term of the patent for the period of

(94) 1,040 days

subject to the payment of maintenance fees as provided by law, with all rights pertaining thereto as provided by 35 U.S.C. § 156.

I have caused the seal of the United States Patent and Trademark Office to be affixed this 14th day of October 2021.

Drew Hirshfeld
Commissioner for Patents, Performing the Functions and Duties of the Under Secretary of Commerce for Intellectual Property and Director of the United States Patent and Trademark Office

EXHIBIT B

US010335462B2

(12) **United States Patent**
Jensen

(10) **Patent No.: US 10,335,462 B2**
(45) **Date of Patent: *Jul. 2, 2019**

(54) **USE OF LONG-ACTING GLP-1 PEPTIDES**

(71) Applicant: **Novo Nordisk A/S**, Bagsvaerd (DK)

(72) Inventor: **Christine Bjoern Jensen**, Charlottenlund (DK)

(73) Assignee: **Novo Nordisk A/S**, Bagsvaerd (DK)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **15/656,042**

(22) Filed: **Jul. 21, 2017**

(65) **Prior Publication Data**

US 2018/0085435 A1 Mar. 29, 2018

Related U.S. Application Data

(63) Continuation of application No. 14/409,493, filed as application No. PCT/EP2013/063004 on Jun. 21, 2013, now Pat. No. 9,764,003.

(60) Provisional application No. 61/708,162, filed on Oct. 1, 2012, provisional application No. 61/694,837, filed on Aug. 30, 2012.

(30) **Foreign Application Priority Data**

Jul. 1, 2012 (EP) 12174535
Oct. 1, 2012 (EP) 12186781

(51) **Int. Cl.**

A61K 38/26	(2006.01)
A61P 5/50	(2006.01)
A61P 3/04	(2006.01)
A61P 3/10	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 38/26*** (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

(56) **References Cited**

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WO	2010/092163	A2	8/2010
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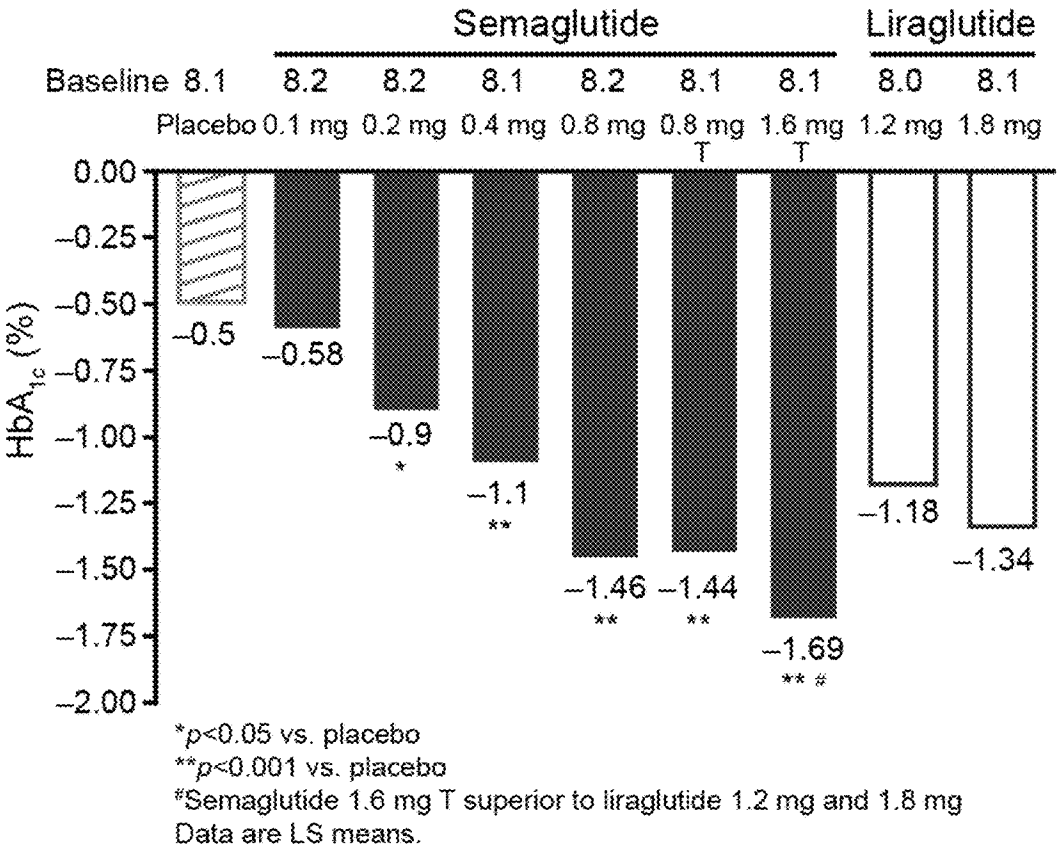
Primary Examiner — Julie Ha
Assistant Examiner — Kristina M Hellman
(74) *Attorney, Agent, or Firm* — Leon Y. Lum

(57) **ABSTRACT**

The invention relates to use of long-acting GLP-1 peptides in certain dosage regimes for the treatment of type 2 diabetes, obesity, etc.

10 Claims, 6 Drawing Sheets

Specification includes a Sequence Listing.



*p<0.05 vs. placebo
**p<0.001 vs. placebo
#Semaglutide 1.6 mg T superior to liraglutide 1.2 mg and 1.8 mg
Data are LS means.

Fig. 1

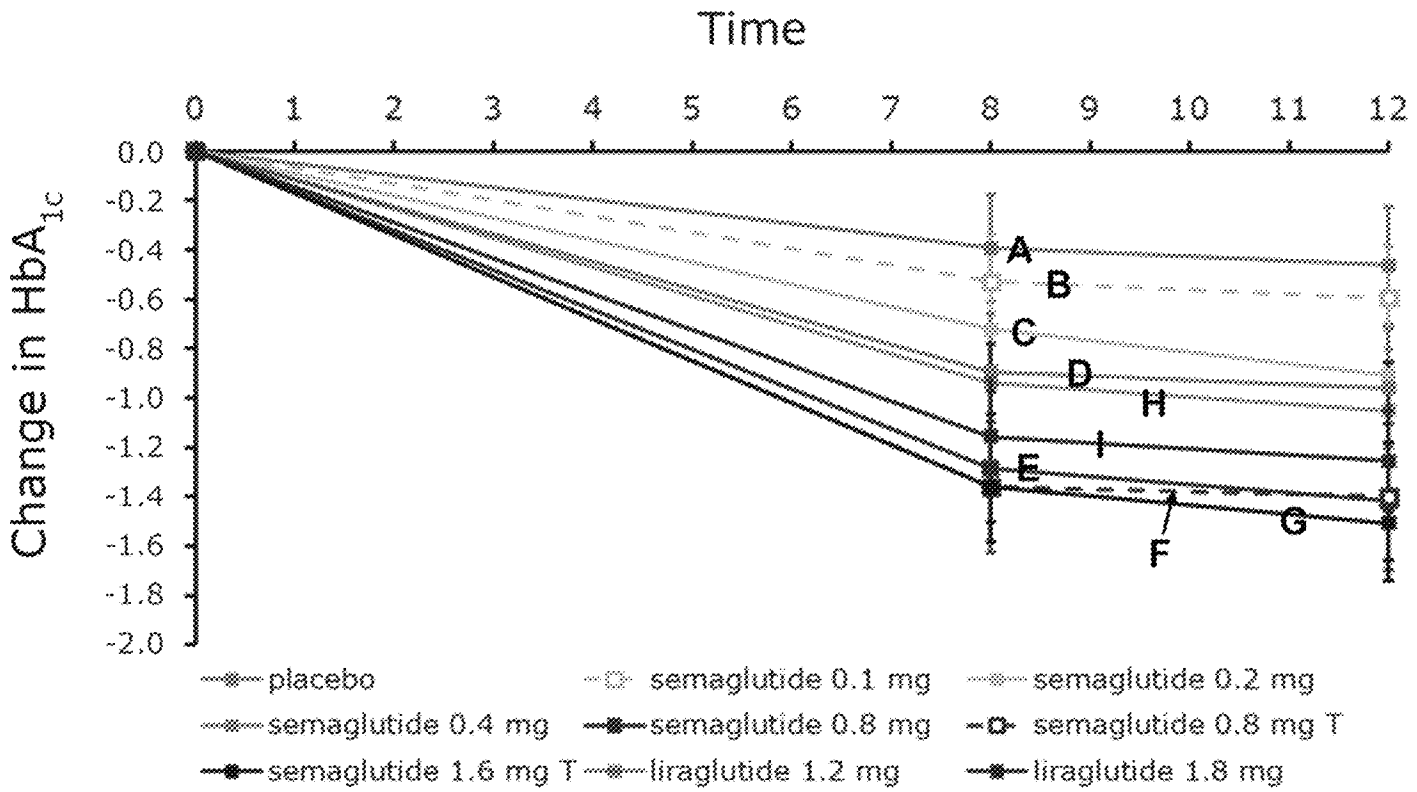

Time
0
1
2
3
4
5
6
7
8
9
10
11
12
Change in HbA1c
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
-2.0
A
B
C
D
H
I
E
F
G
placebo
semaglutide 0.1 mg
semaglutide 0.2 mg
semaglutide 0.4 mg
semaglutide 0.8 mg
semaglutide 0.8 mg T
semaglutide 1.6 mg T
liraglutide 1.2 mg
liraglutide 1.8 mg

Fig. 2

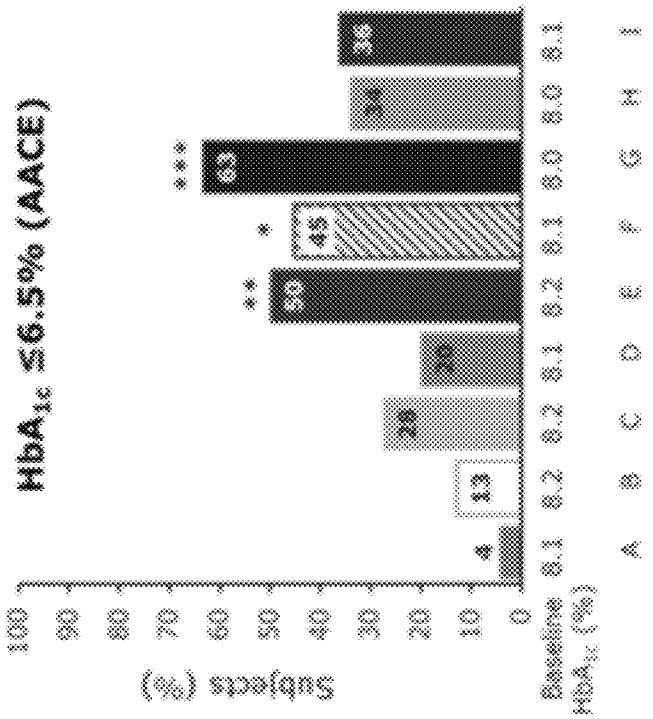

HbA1c ≤6.5% (AACE)
Subjects (%)
100
90
80
70
60
50
40
30
20
10
0
4
13
28
29
50
43
63
***
*
**
A
B
C
D
E
F
G
H
I
Baseline HbA1c (%)
8.1
8.2
8.2
8.1
8.2
8.1
8.0
8.0
8.1

Fig. 3A

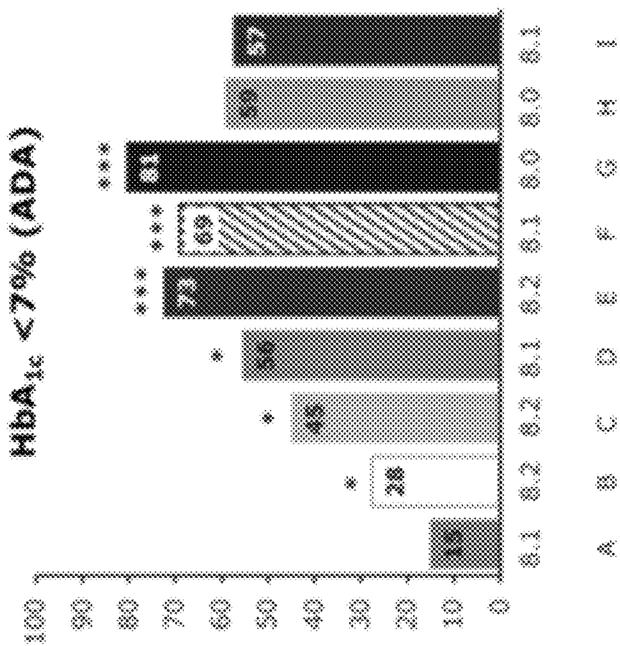


Fig. 3 B

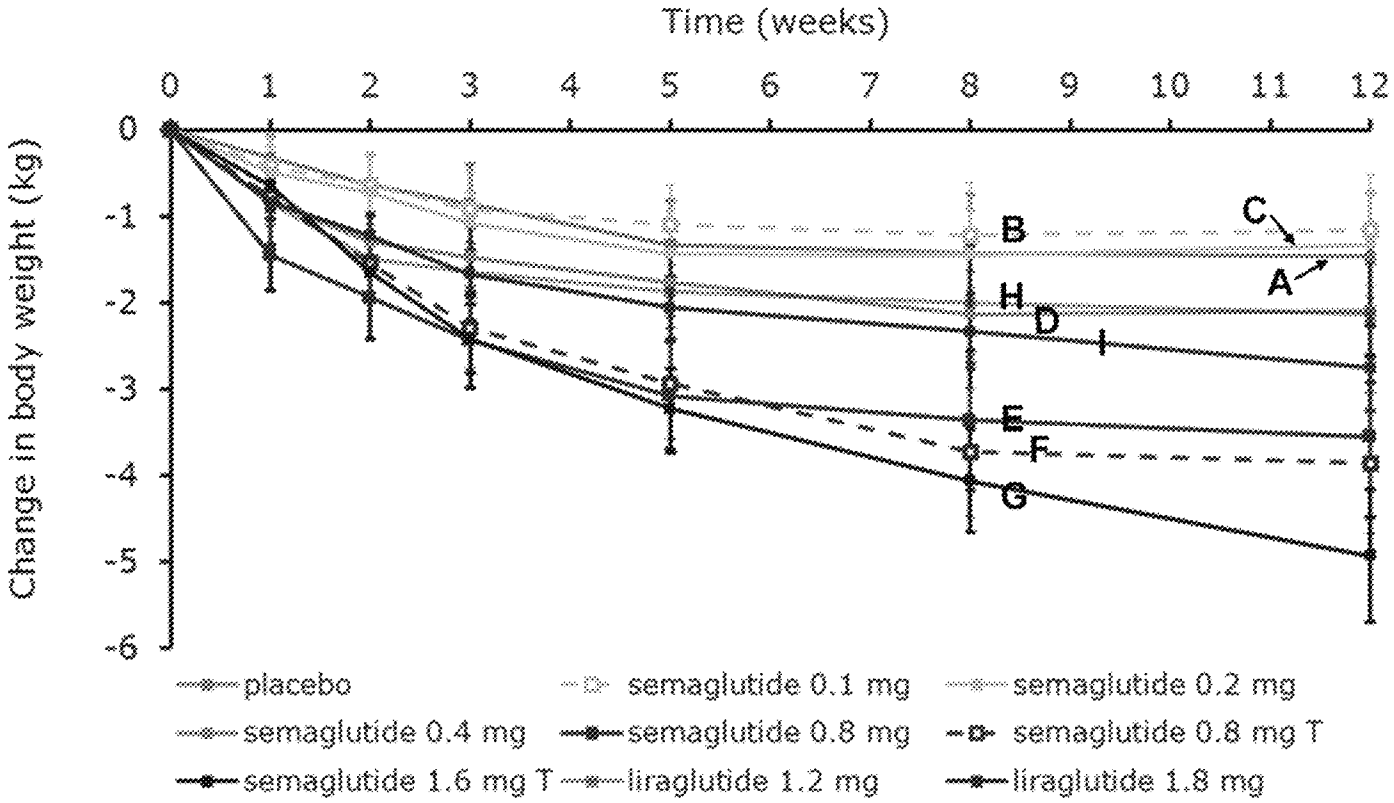

Time (weeks)
0
1
2
3
4
5
6
7
8
9
10
11
12
Change in body weight (kg)
0
-1
-2
-3
-4
-5
-6
A
B
C
D
E
F
G
H
placebo
semaglutide 0.1 mg
semaglutide 0.2 mg
semaglutide 0.4 mg
semaglutide 0.8 mg
semaglutide 0.8 mg T
semaglutide 1.6 mg T
liraglutide 1.2 mg
liraglutide 1.8 mg

Fig. 4

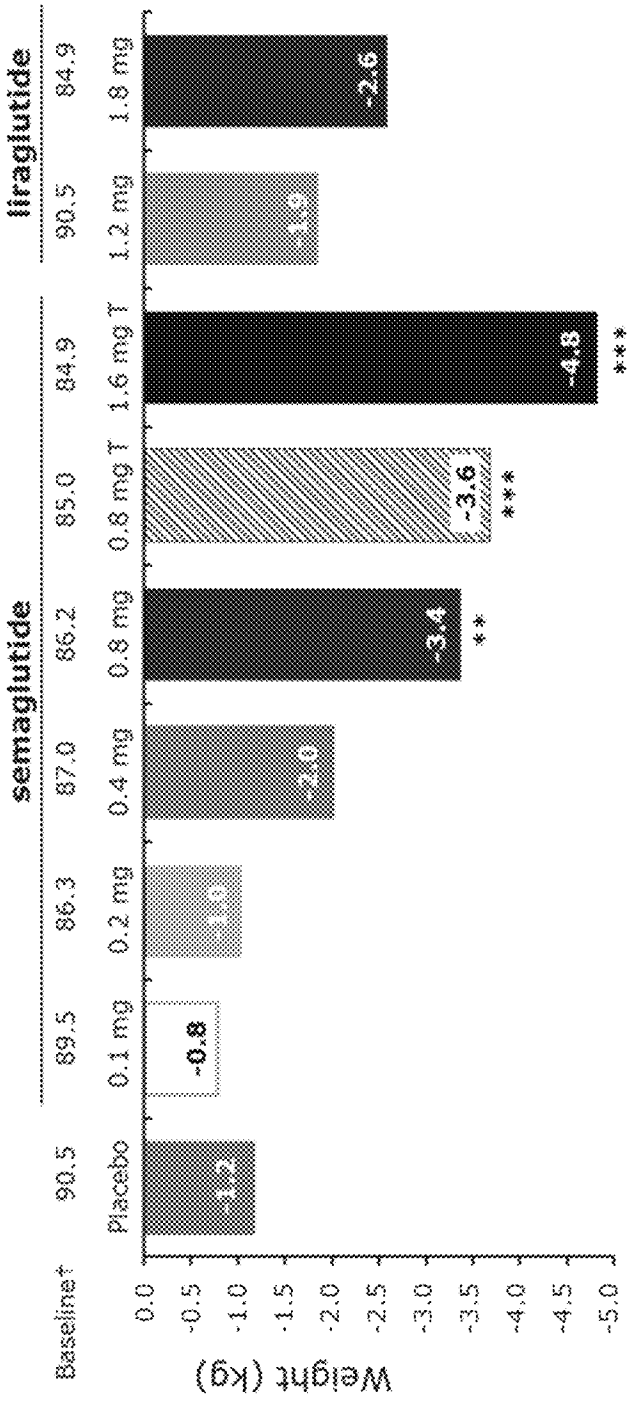

Weight (kg)
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
-4.0
-4.5
-5.0
Baseline†
90.5
Placebo
-1.2
89.5
0.1 mg
-0.8
86.3
0.2 mg
-1.0
87.0
0.4 mg
-2.0
86.2
0.8 mg
-3.4
**
85.0
0.8 mg T
-3.6
***
84.9
1.6 mg T
-4.8
***
semaglutide
90.5
1.2 mg
-1.9
84.9
1.8 mg
-2.6
liraglutide

Fig. 5

USE OF LONG-ACTING GLP-1 PEPTIDES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. application Ser. No. 14/409,493, filed Dec. 19, 2014, which is a 35 U.S.C. § 371 National Stage application of International Application PCT/EP2013/063004 (WO 2014/005858), filed Jun. 21, 2013, which claimed priority of European Patent Application 12174535.0, filed Jul. 1, 2012 and European Patent Application 12186781.6, filed Oct. 1, 2012; this application claims priority under 35 U.S.C. § 119 of U.S. Provisional Application 61/694,837; filed Aug. 30, 2012 and U.S. Provisional Application 61/708,162; filed Oct. 1, 2012.

The present invention relates to improved uses of GLP-1 peptides in therapy.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Jun. 17, 2013 and amended on Jul. 12, 2017, is named 8545US02_SeqList.txt and is 7,975 bytes in size.

SUMMARY

In one embodiment the invention relates to a method for a) reduction of HbA1c; b) prevention or treatment of type 2 diabetes, hyperglycemia, impaired glucose tolerance, or non-insulin dependent diabetes; or c) prevention or treatment of obesity, reducing body weight and/or food intake, or inducing satiety; wherein said method comprises administration of a GLP-1 agonist to a subject in need thereof, wherein said GLP-1 agonist i) has a half-life of at least 72 hours, wherein said half-life optionally is determined by Assay (II); ii) is administered in an amount of at least 0.7 mg per week, such an amount equivalent to at least 0.7 mg semaglutide per week; and iii) is administered once weekly or less often.

In one embodiment the invention relates to a GLP-1 agonist for use in a) the reduction of HbA1c; b) the prevention or treatment of type 2 diabetes, hyperglycemia, impaired glucose tolerance, or non-insulin dependent diabetes; or c) the prevention or treatment of obesity, for reducing body weight and/or food intake, or for inducing satiety; wherein said use comprises administration of said GLP-1 agonist in an amount of at least 0.7 mg per week, such an amount equivalent to at least 0.7 mg semaglutide per week, and wherein said GLP-1 agonist and/or administration optionally is as defined herein.

In one embodiment the invention relates to a composition comprising a GLP-1 agonist for use in a) the reduction of HbA1c; b) the prevention or treatment of type 2 diabetes, hyperglycemia, impaired glucose tolerance, or non-insulin dependent diabetes; or c) the prevention or treatment of obesity, for reducing body weight and/or food intake, or for inducing satiety; wherein said GLP-1 agonist i) has a half-life of at least 72 hours, wherein said half-life optionally is determined by Assay (II); and ii) is administered in an amount of at least 0.7 mg per week, such an amount equivalent to at least 0.7 mg semaglutide per week; and wherein said composition is administered once weekly or less often, and wherein said GLP-1 agonist and/or administration optionally is as defined herein.

BRIEF DESCRIPTION OF DRAWINGS

FIG. **1** shows change in HbA1c following subcutaneous administration of placebo, semaglutide, or liraglutide to human subjects. *p<0.05 vs. placebo; **p<0.001 vs. placebo (based on adjusted means). Baseline values are for information only: data are model-adjusted for baseline HbA1c. Data are model-adjusted LS means, FAS LOCF. The estimates are from an ANOVA model with treatment, country and previous treatment as fixed effects and baseline HbA1c as covariate.

FIG. **2** shows mean change in HbA1c from baseline versus time; data are mean (1.96SE), FAS LOCF. The treatments are placebo (A); semaglutide 0.1 mg (B, dashed line), 0.2 mg (C), 0.4 mg (D), 0.8 mg (E), 0.8 mg T (F, dashed line), 1.6 mg T (G); liraglutide 1.2 mg (H), 1.8 mg (I).

FIG. **3**A and FIG. **3**B show subjects reaching the AACE (FIG. **3**A) or ADA (FIG. **3**B) criteria for glycaemic control. The number of patients reaching the criteria per treatment is indicated in each bar. The treatments are placebo (A); semaglutide 0.1 mg (B), 0.2 mg (C), 0.4 mg (D), 0.8 mg (E), 0.8 mg T (F), 1.6 mg T (G); liraglutide 1.2 mg (H), 1.8 mg (I). *p<0.05 vs. placebo; **p<0.001 vs. placebo; ***p<0.0001 vs. placebo (based on adjusted means). Data are FAS LOCF. The estimates are from a logistic regression model treatment, country and previous treatment as fixed effects and baseline HbA1c as covariate. ADA, American Diabetes Association; AACE, American Association of Clinical Endocrinologists.

FIG. **4** shows mean body weight change versus time; data are mean (1.96SE), FAS LOCF. The treatments are placebo (A); semaglutide 0.1 mg (B, dashed line), 0.2 mg (C), 0.4 mg (D), 0.8 mg (E), 0.8 mg T (F, dashed line), 1.6 mg T (G); liraglutide 1.2 mg (H), 1.8 mg (I).

FIG. **5** shows body weight change from baseline at week 12. **p<0.001 vs. placebo; ***p<0.0001 vs. placebo (based on adjusted means. †: Baseline values for information only: data are model-adjusted for baseline weight. Data are model-adjusted LS means, FAS LOCF. The estimates are from an ANOVA model with treatment, country and previous treatment as fixed effects and baseline weight as covariate.

SE: Standard error. FAS: Full analysis set. LOCF: Last observation carried forward.

DESCRIPTION

The present invention relates to an improved use of GLP-1 agonists in therapy. In one embodiment the invention relates to certain dosage regimes of GLP-1 agonists which provide improved effect in diseases or conditions, such as prevention and/or treatment of type 2 diabetes and obesity. In one embodiment the methods of the present invention provides surprisingly showed improved reduction of HbA1c and reduction of body weight. In one embodiment the GLP-1 agonist is administered in an amount which provides an improved a) reduction in HbA1c or b) reduction in body weight compared to administration of 1.8 mg liraglutide or less, such as 0.8 mg liraglutide or less, per day.

In one embodiment the invention relates to a method for reduction of HbA1c or for prevention or treatment of type 2 diabetes, hyperglycemia, impaired glucose tolerance, or non-insulin dependent diabetes, said method comprising administration of a GLP-1 agonist to a subject in need

thereof in an amount of at least 0.7 mg per week, such an amount equivalent to at least 0.7 mg semaglutide per week. In one embodiment the method is for reduction of HbA1c. In one embodiment the method is for prevention or treatment of type 2 diabetes. In one embodiment the method is for prevention or treatment of hyperglycemia. In one embodiment the method is for prevention or treatment of impaired glucose tolerance. In one embodiment the method is for prevention or treatment of non-insulin dependent diabetes. In one embodiment the method of the invention comprises delaying or preventing diabetic disease progression. In one embodiment a HbA1c level below 7% is achieved. In one embodiment the level of HbA1c is determined according to the method defined by the Diabetes Control and Complications Trial (DCCT). In one embodiment the level of HbA1c is determined according to the method defined by the International Federation of Clinical Chemistry (IFCC).

In one embodiment the invention relates to a method for treating or preventing obesity, for reducing body weight and/or food intake, or for inducing satiety, said method comprising administration of a GLP-1 agonist to a subject in need thereof in an amount of at least 0.7 mg per week, such an amount equivalent to at least 0.7 mg semaglutide per week. In one embodiment the method is for prevention or treatment of obesity. In one embodiment the method is for reducing body weight and/or food intake. In one embodiment the method is for inducing satiety.

In one embodiment the GLP-1 agonist has a half-life of at least 24 hours, such as at least 48 hours, at least 60 hours, or at least 72 hours, or such as at least 84 hours, at least 96 hours, or at least 108 hours, or optionally at least 120 hours, at least 132 hours, or at least 144 hours, wherein said half-life optionally is determined by Assay (II).

In one embodiment the GLP-1 agonist is administered twice weekly or less often, once weekly or less often, or once weekly or less often. In one embodiment the GLP-1 agonist is administered once every secondly week or less often, once every third week or less often, or once a month or less often.

In one embodiment the GLP-1 agonist is administered in an amount per week of at least 0.8 mg, at least 0.9 mg, or at least 1.0 mg. In one embodiment the GLP-1 agonist is administered in an amount per week of at least 1.1 mg, at least 1.2 mg, or at least 1.3 mg. In one embodiment the GLP-1 agonist is administered in an amount per week of at least 1.4 mg, at least 1.5 mg, or at least 1.6 mg.

In one embodiment the GLP-1 agonist is administered in an amount per week equivalent to at least 0.8 mg, at least 0.9 mg, or at least 1.0 mg semaglutide. In one embodiment the GLP-1 agonist is administered in an amount per week equivalent to at least 1.1 mg, at least 1.2 mg, or at least 1.3 mg semaglutide. In one embodiment the GLP-1 agonist is administered in an amount per week equivalent to at least 1.4 mg, at least 1.5 mg, or at least 1.6 mg semaglutide.

In one embodiment the GLP-1 agonist is selected from the group consisting of semaglutide, exenatide, albiglutide, and dulaglutide.

In one embodiment the GLP-1 agonist is administered by parenteral administration, such as subcutaneous injection.

In one embodiment the GLP-1 agonist is a GLP-1 peptide. In one embodiment the GLP-1 peptide comprises no more than 5, such as no more than 4 or no more than 3, amino acid residues which have been substituted, inserted or deleted as compared to GLP-1 (7-37). In one embodiment the GLP-1 peptide comprises no more than 4 amino acid residues which are not encoded by the genetic code.

In one embodiment the GLP-1 peptide is a DPPIV protected GLP-1 peptide. In one embodiment the GLP-1 peptide is DPPIV stabilised.

In one embodiment the GLP-1 agonist has an EC_{50} at or below 3000 pM, such as at or below 500 pM or at or below 100 pM, optionally determined by Assay (I).

In one embodiment the invention relates to a GLP-1 agonist for use in the reduction of HbA1c or for use in the prevention or treatment of type 2 diabetes, hyperglycemia, impaired glucose tolerance, or non-insulin dependent diabetes comprising administering a GLP-1 agonist in an amount of at least 0.7 mg per week, such an amount equivalent to at least 0.7 mg semaglutide per week. In one embodiment the GLP-1 agonist and/or administration is as defined herein.

In one embodiment the invention relates to a GLP-1 agonist for use in the prevention or treatment of obesity, in the reduction of body weight and/or food intake, or in the induction satiety comprising administering a GLP-1 agonist in an amount of at least 0.7 mg per week, such an amount equivalent to at least 0.7 mg semaglutide per week. In one embodiment the GLP-1 agonist and/or administration is as defined herein.

In one embodiment the invention relates to a composition comprising a GLP-1 agonist and one or more pharmaceutically acceptable excipients for use in reduction of HbA1c or for prevention or treatment of type 2 diabetes, hyperglycemia, impaired glucose tolerance, or non-insulin dependent diabetes, wherein said GLP-1 agonist is administered in an amount of at least 0.7 mg per week, such an amount equivalent to at least 0.7 mg semaglutide per week. In one embodiment the GLP-1 agonist and/or administration is as defined herein.

In one embodiment the invention relates to a composition comprising a GLP-1 agonist and one or more pharmaceutically acceptable excipients for use in the prevention or treatment of obesity, in the reduction of body weight and/or food intake, or in the induction satiety, wherein said GLP-1 agonist is administered in an amount of at least 0.7 mg per week, such an amount equivalent to at least 0.7 mg semaglutide per week. In one embodiment the GLP-1 agonist and/or administration is as defined herein.

In one embodiment the GLP-1 agonist is administered with another therapeutic agent. Administration with another therapeutic agent may be carried out as administration of the GLP-1 agonist and the other therapeutic agent within the same therapeutic window (e.g. within a period of two weeks, a period of one week, or in a 96, 72, or 48 hour period, etc.). The treatment with a GLP-1 agonist according to the present invention may be combined with one or more additional therapeutic agents, e.g. selected from antidiabetic agents, antiobesity agents, appetite regulating agents, antihypertensive agents, agents for the treatment and/or prevention of complications resulting from or associated with diabetes and agents for the treatment and/or prevention of complications and disorders resulting from or associated with obesity; examples of these therapeutic agents are: sulphonylureas, thiazolidinediones, biguanides, meglitinides, glucosidase inhibitors, glucagon antagonists, and DPP-IV (dipeptidyl peptidase-IV) inhibitors.

In one embodiment, as used herein, an "amount equivalent to" when used in relation to GLP-1 agonists refers to amounts of a first GLP-1 agonist and a second GLP-1 agonist having GLP-1 receptor potency (i.e. EC_{50}) within ±30%, such as within ±20% or within ±10%, of each other optionally determined by Assay (I) described herein and

having a half-life within ±30%, such as within ±20% or within ±10%, of each other optionally determined by Assay (II) described herein.

In one embodiment an "effective amount" of a GLP-1 agonist as used herein means an amount sufficient to cure, alleviate, or partially arrest the clinical manifestations of a given disease or state and its complications. An amount adequate to accomplish this is defined as "effective amount". Effective amounts for each purpose will depend on the severity of the disease or injury as well as the weight and general state of the subject. It will be understood that determining an appropriate dosage may be achieved using routine experimentation, by constructing a matrix of values and testing different points in the matrix, which is all within the ordinary skills of a trained physician or veterinary.

In one embodiment the term "treatment" or "treating" as used herein means the management and care of a patient for the purpose of combating a condition, such as a disease or a disorder. In one embodiment the term "treatment" or "treating" is intended to include the full spectrum of treatments for a given condition from which the patient is suffering, such as administration of the active compound to alleviate the symptoms or complications; to delay the progression of the disease, disorder, or condition; to alleviate or relieve the symptoms and complications; and/or, to cure or eliminate the disease, disorder, or condition as well as to prevent the condition. In one embodiment prevention is to be understood as the management and care of a patient for the purpose of combating the disease, condition, or disorder and includes the administration of the active compounds to prevent the onset of the symptoms or complications.

In one embodiment the term "hydrophilic spacer" as used herein means a spacer that separates a peptide and an albumin binding residue with a chemical moiety which comprises at least 5 non-hydrogen atoms where 30-50% of these are either N or O.

In one embodiment the term "analogue" as used herein referring to a polypeptide means a modified peptide wherein one or more amino acid residues of the peptide have been substituted by other amino acid residues and/or wherein one or more amino acid residues have been deleted from the peptide and or wherein one or more amino acid residues have been added to the peptide. Such addition or deletion of amino acid residues can take place at the N-terminal of the peptide and/or at the C-terminal of the peptide. A simple system is used to describe analogues: For example Arg^{34}GLP-1 (7-37) Lys designates a GLP-1 analogue wherein the naturally occurring lysine at position 34 has been substituted with arginine and a lysine residue has been added to the C-terminal (position 38).

In one embodiment the term "GLP-1 peptide" as used herein means GLP-1 (7-37), a GLP-1 analogue, a GLP-1 derivative or a derivative of a GLP-1 analogue.

In one embodiment the term "exendin-4 peptide" as used herein means exendin-4 (1-39), an exendin-4 analogue, an exendin-4 derivative or a derivative of an exendin-4 analogue.

In one embodiment the term "DPP-IV protected" as used herein referring to a polypeptide means a polypeptide which has been chemically modified in order to render said compound resistant to the plasma peptidase dipeptidyl aminopeptidase-4 (DPP-IV). The DPP-IV enzyme in plasma is known to be involved in the degradation of several peptide hormones, e.g. GLP-1, Exendin-4 etc. Thus a considerable effort is being made to develop GLP-1 agonists less susceptible to DPP-IV mediated hydrolysis in order to reduce the rate of degradation by DPP-IV.

The present invention also relates to a GLP-1 agonist of the invention, for use as a medicament. In particular embodiments, the GLP-1 agonist of the invention may be used for the following medical treatments:

(i) prevention and/or treatment of all forms of diabetes, such as hyperglycemia, type 2 diabetes, impaired glucose tolerance, type 1 diabetes, non-insulin dependent diabetes, MODY (maturity onset diabetes of the young), gestational diabetes, and/or for reduction of HbA1c;

(ii) delaying or preventing diabetic disease progression, such as progression in type 2 diabetes, delaying the progression of impaired glucose tolerance (IGT) to insulin requiring type 2 diabetes, and/or delaying the progression of non-insulin requiring type 2 diabetes to insulin requiring type 2 diabetes;

(iii) prevention and/or treatment of eating disorders, such as obesity, e.g. by decreasing food intake, reducing body weight, suppressing appetite, inducing satiety; treating or preventing binge eating disorder, bulimia nervosa, and/or obesity induced by administration of an antipsychotic or a steroid; reduction of gastric motility; and/or delaying gastric emptying.

In another particular embodiment, the indication is (i). In a further particular embodiment the indication is (ii). In a still further particular embodiment the indication is (iii). In one embodiment the indication is type 2 diabetes and/or obesity.

In one embodiment the method comprises prevention, treatment, reduction and/or induction in one or more diseases or conditions defined herein. In one embodiment the indication is (i) and (iii). In one embodiment the indication is (ii) and (iii). In one embodiment the method comprises prevention, treatment, reduction and/or induction in one or more diseases or conditions selected from a) and b), a) and c), b) and c), or a), b) and c) as defined in claim **1**.

In one embodiment the invention relates to administration of an effective amount of a GLP-1 agonist.

In one embodiment as used herein, specific values given in relation to numbers or intervals may be understood as the specific value or as about the specific value.

Functional Properties

In a first functional aspect, the GLP-1 agonists of the invention have a good potency. Also, or alternatively, in a second functional aspect, the GLP-1 agonists of the invention have a protracted pharmacokinetic profile. Also, or alternatively, in a third functional aspect, the GLP-1 agonists of the invention are stable against degradation by gastro intestinal enzymes.

Biological Activity (Potency)

According to the first functional aspect, the GLP-1 agonists of the invention are biologically active, or potent. In a particular embodiment, "potency" and/or "activity" refers to in vitro potency, i.e. performance in a functional GLP-1 receptor assay, more in particular to the capability of stimulating cAMP formation in a cell line expressing the cloned human GLP-1 receptor.

The stimulation of the formation of cAMP in a medium containing the human GLP-1 receptor may preferably be determined using a stable transfected cell-line such as BHK467-12A (tk-ts13), and/or using for the determination of cAMP a functional receptor assay, e.g. based on competition between endogenously formed cAMP and exogenously added biotin-labelled cAMP, in which assay cAMP is more preferably captured using a specific antibody, and/or wherein an even more preferred assay is the AlphaScreen cAMP Assay, such as the one described in Assay (I).

In one embodiment the term half maximal effective concentration (EC_{50}) generally refers to the concentration which induces a response halfway between the baseline and maximum, by reference to the dose response curve. EC_{50} is used as a measure of the potency of a compound and represents the concentration where 50% of its maximal effect is observed.

The in vitro potency of the GLP-1 agonists of the invention may be determined as described above, and the EC_{50} of the GLP-1 agonist in question determined. The lower the EC_{50}, the better the potency.

In a particular embodiment, the medium has the following composition (final in-assay concentrations): 50 mM TRIS-HCl; 5 mM HEPES; 10 mM $MgCl_2$, $6H_2O$; 150 mM NaCl; 0.01% Tween; 0.1% BSA; 0.5 mM IBMX; 1 mM ATP; 1 μM GTP; pH 7.4.

In a further particular embodiment, the GLP-1 agonist of the invention has an in vitro potency corresponding to an EC_{50} at or below 3000 pM, such as below 2000 pM, below 1000 pM, or below 500 pM, or such as below 200 pM or below 100 pM.

In another particular embodiment the GLP-1 agonist of the invention are potent in vivo, which may be determined as is known in the art in any suitable animal model, as well as in clinical trials.

The diabetic db/db mouse is one example of a suitable animal model, and the blood glucose lowering effect may be determined in such mice in vivo, e.g. as described in Assay (III), or as described in Example 43 of WO09/030738.

Also, or alternatively, the effect on food intake in vivo may be determined in pharmacodynamic studies in pigs, e.g. as described in Assay (IV).

Protraction—Half Life In Vivo in Minipigs

According to the second functional aspect, the GLP-1 agonists of the invention are protracted. In a particular embodiment protraction may be determined as half-life ($T_{1/2}$) in vivo in minipigs after i.v. administration. In additional embodiments, the half-life is at least 24 hours, such as at least 48 hours, at least 60 hours, at least 72 hours, or such as at least 84 hours, at least 96 hours, or at least 108 hours.

A suitable assay for determining half-life in vivo in minipigs after i.v. administration is disclosed in Assay (II).

Degradation by Gastro Intestinal Enzymes

According to the third functional aspect, the GLP-1 agonists of the invention are stable, or stabilised, against degradation by one or more gastro intestinal enzymes.

Gastro intestinal enzymes include, without limitation, exo and endo peptidases, such as pepsin, trypsin, chymotrypsin, elastases, and carboxypeptidases. The stability may be tested against these gastro intestinal enzymes in the form of purified enzymes, or in the form of extracts from the gastrointestinal system.

In a particular embodiment, the GLP-1 agonist of the invention has an in vitro half-life ($T_{1/2}$), in an extract of rat small intestines, divided by the corresponding half-life ($T_{1/2}$) of GLP-1(7-37), of at least 1, such as above 1.0, at least 1.2, at least 2.0, or such as at least 3.0, or at least 4.0. In other words, a ratio (SI) may be defined for each GLP-1 agonist, viz. as the in vitro half-life ($T_{1/2}$) of the GLP-1 agonist in question, in an extract of rat small intestines, divided by the corresponding half-life ($T_{1/2}$) of GLP-1(7-37).

A suitable assay for determining in vitro half-life in an extract of rat small intestines is disclosed in Assay (V).

GLP-1 Agonists

In one embodiment the GLP-1 peptide comprises an Aib residue in position 8.

In one embodiment the amino acid residue in position 7 of said GLP-1 peptide is selected from the group consisting of D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, N^{α}-acetyl-histidine, α-fluoromethyl-histidine, α-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine and 4-pyridylalanine.

In one embodiment the GLP-1 peptide is attached to a hydrophilic spacer via the amino acid residue in position 23, 26, 34, 36 or 38 relative to the amino acid sequence of GLP-1 (7-37).

In one embodiment the GLP-1 peptide is exendin-4, an exendin-4-analogue, or a derivative of exendin-4.

In one embodiment the GLP-1 agonist peptide comprises the amino acid sequence of the following formula:

H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH_2 (SEQ ID NO: 2).

In one embodiment the GLP-1 agonist comprises an albumin binding residue attached via a hydrophilic spacer to the C-terminal amino acid residue of said GLP-1 peptide.

In one embodiment the GLP-1 agonist comprises a second albumin binding residue is attached to an amino acid residue which is not the C-terminal amino acid residue.

In one embodiment the GLP-1 peptide is selected from the group consisting of semaglutide, albiglutide and dulaglitide.

In one embodiment the GLP-1 peptide has the following structure:

His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Aib-Arg (SEQ ID NO: 3).

In one embodiment the GLP-1 peptide has the following structure:

(His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg)2 (SEQ ID NO: 4)

genetically fused to human albumin.

In one embodiment the GLP-1 peptide is dulaglitide.

In one embodiment the GLP-1 agonists of the invention have GLP-1 activity. In one embodiment "a GLP-1 agonist" is understood to refer to any compound, including peptides and non-peptide compounds, which fully or partially activate the human GLP-1 receptor. In one embodiment the "GLP-1 agonist" is any peptide or non-peptide small molecule that binds to a GLP-1 receptor, preferably with an affinity constant (K_D) or a potency (EC_{50}) of below 1 μM, e. g. below 100 nM as measured by methods known in the art (see e. g., WO 98/08871). In one embodiment methods for identifying GLP-1 agonists are described in WO 93/19175 (Novo Nordisk A/S) and examples of suitable GLP-1 agonists which can be used according to the present invention includes those referred to in WO 2005/027978 (Novo Nordisk A/S), the teachings of which are both incorporated by reference herein. "GLP-1 activity" refers to the ability to bind to the GLP-1 receptor and initiate a signal transduction pathway resulting in insulinotropic action or other physiological effects as is known in the art. For example, the GLP-1 agonists of the invention can be tested for GLP-1 activity using the assay described in Assay (I) herein.

In yet another embodiment the GLP-1 agonist is a stable GLP-1 agonist. As used herein a "stable GLP-1 agonist" means a GLP-1 agonist which exhibits an in vivo plasma elimination half-life of at least 24 hours in man, optionally

determined by the method described below. Examples of stable GLP-1 agonists can be found in WO02006/097537.

In one embodiment the method for determination of plasma elimination half-life of a compound in man may be carried out as follows: The compound is dissolved in an isotonic buffer, pH 7.4, PBS or any other suitable buffer. The dose is injected peripherally, preferably in the abdominal or upper thigh. Blood samples for determination of active compound are taken at frequent intervals, and for a sufficient duration to cover the terminal elimination part (e. g., Predose, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24 (day 2), 36 (day 2), 48 (day 3), 60 (day 3), 72 (day 4) and 84 (day 4) hours post dose). Determination of the concentration of active compound is performed as described in Wilken et al., Diabetologia 43 (51), 2000. Derived pharmacokinetic parameters are calculated from the concentration-time data for each individual subject by use of non-compartmental methods, using the commercially available software WinNonlin Version 2.1 (Pharsight, Cary, N.C., USA). The terminal elimination rate constant is estimated by log-linear regression on the terminal log-linear part of the concentration-time curve, and used for calculating the elimination half-life.

In one embodiment the GLP-1 agonist is formulated so as to have a half-life in man of at least 48 hours. This may be obtained by sustained release formulations known in the art.

In one embodiment the GLP-1 agonist is a GLP-1 peptide. In one embodiment the GLP-1 peptide is selected from GLP-1 (7-35), GLP-1 (7-36), GLP-1 (7-36)-amide, GLP-1 (7-37), GLP-1 (7-38), GLP-1 (7-39), GLP-1 (7-40), GLP-1 (7-41) or an analogue or derivative thereof. In one embodiment the GLP-1 peptide comprises no more than 15, such as no more than 10 or no more than 6, amino acid residues which have been substituted, inserted or deleted as compared to GLP-1 (7-37). In one embodiment the GLP-1 peptide comprises no more than 4 amino acid residues which are not encoded by the genetic code. In yet another embodiment, the GLP-1 agonist is exendin-4 or exendin-3, an exendin-4 or exendin-3 analogue, or a derivative of any of these.

In one embodiment the GLP-1 peptide is selected from the group consisting of semaglutide, exenatide, albiglutide, and dulaglitide. In one embodiment the GLP-1 peptide is semaglutide. WO 06/097537 discloses semaglutide (Example 4), a mono-acylated GLP-1 agonist for once weekly administration. In one embodiment the GLP-1 peptide is exenatide. In one embodiment the GLP-1 peptide comprises the amino acid sequence of the formula: H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH_2 (SEQ ID NO: 2). Exenatide is a synthetic version of exendin-4, a hormone found in the saliva of the Gila monster. Exenatide displays biological properties similar to GLP-1. In some embodiments the composition is BYDUREON® (a long acting release formula of exenatide in PLGA particles). In one embodiment the "Bydureon® composition" refer to a powder comprising exenatide, poly (D,L-lactide-co-glycolide), and sucrose which immediately prior to injection is reconstituted in a solvent comprising carmellose sodium, sodium chloride, polysorbate 20, monobasic sodium phosphate (e.g. its monohydrate), dibasic sodium phosphate (e.g. its heptahydrate), and water for injections. In one embodiment the GLP-1 peptide has the structure (His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg)2 (SEQ ID NO: 4)-genetically fused to human albumin. Albiglutide is a recombinant human serum albumin (HSA)-GLP-1 hybrid protein, likely a GLP-1 dimer fused to HSA. The constituent GLP-1 peptide is analogue, in which Ala at position 8 has been substituted by Glu. In one embodiment the GLP-1 peptide is dulaglitide. Dulaglutide is a GLP-1-Fc construct (GLP-1-linker-Fc from IgG4). In one embodiment the GLP-1 peptide has the structure His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phelle-Ala-Trp-Leu-Val-Lys-Aib-Arg (SEQ ID NO: 3). Liraglutide is a mono-acylated GLP-1 agonist for once daily administration which is marketed as of 2009 by Novo Nordisk A/S, is disclosed in WO 98/08871 Example 37.

In one embodiment the present invention encompasses pharmaceutically acceptable salts of the GLP-1 agonists. Such salts include pharmaceutically acceptable acid addition salts, pharmaceutically acceptable metal salts, ammonium, and alkylated ammonium salts. Also intended as pharmaceutically acceptable acid addition salts are the hydrates which the present GLP-1 agonists are able to form.

In one embodiment the route of administration of GLP-1 agonists may be any route which effectively transports the active compound to the appropriate or desired site of action, such as parenteral. In one embodiment medicaments or pharmaceutical compositions comprising a GLP-1 agonist, such as semaglutide, may be administered parenterally to a patient in need thereof. In one embodiment parenteral administration may be performed by subcutaneous, intramuscular or intravenous injection by means of a syringe, optionally a pen-like syringe.

Alternatively, parenteral administration can be performed by means of an infusion pump. A further option is a composition which may be a powder or a liquid for the administration of a GLP-1 agonist in the form of a nasal or pulmonal spray. As a still further option, the GLP-1 agonist can also be administered transdermally, e.g., from a patch, optionally an iontophoretic patch, or transmucosally, e.g., bucally. The above-mentioned possible ways to administer GLP-1 agonists are not considered as limiting the scope of the invention.

In one embodiment the GLP-1 agonist is co-administered together with a further therapeutically active agent used in the treatments defined herein.

In one embodiment the GLP-1 peptide comprises the amino acid sequence of the formula (I) (SEQ ID NO: 5):

Formula (I)

Xaa_7-Xaa_8-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Xaa_{16}-Ser-

Xaa_{18}-$Xaa_{19}$$Xaa_{20}$GluXaa$_{22}$-$Xaa_{23}$-Ala-$Xaa_{25}$-$Xaa_{26}$-$Xaa_{27}$-

Phe-Ile-Xaa_{30}-Trp-Leu-Xaa_{33}-Xaa_{34}-Xaa_{35}-Xaa_{36}-Xaa_{37}-

Xaa_{38}-Xaa_{39}-Xaa_{40}-Xaa_{41}-Xaa_{42}-Xaa_{43}-Xaa_{44}-Xaa_{45}-Xaa_{46}

wherein

- Xaa_7 is L-histidine, D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, N^{α}-acetyl-histidine, α-fluoromethyl-histidine, α-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine;
- Xaa_8 is Ala, Gly, Val, Leu, Ile, Lys, Aib, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid;
- Xaa_{16} is Val or Leu;
- Xaa_{18} is Ser, Lys or Arg;

Xaa_{19} is Tyr or Gln;
Xaa_{20} is Leu or Met;
Xaa_{22} is Gly, Glu or Aib;
Xaa_{23} is Gln, Glu, Lys or Arg;
Xaa_{25} is Ala or Val;
Xaa_{26} is Lys, Glu or Arg;
Xaa_{27} is Glu or Leu;
Xaa_{30} is Ala, Glu or Arg;
Xaa_{33} is Val or Lys;
Xaa_{34} is Lys, Glu, Asn or Arg;
Xaa_{35} is Gly or Aib;
Xaa_{36} is Arg, Gly or Lys;
Xaa_{37} is Gly, Ala, Glu, Pro, Lys, amide or is absent;
Xaa_{38} is Lys, Ser, amide or is absent;
Xaa_{39} is Ser, Lys, amide or is absent;
Xaa_{40} is Gly, amide or is absent;
Xaa_{41} is Ala, amide or is absent;
Xaa_{42} is Pro, amide or is absent;
Xaa_{43} is Pro, amide or is absent;
Xaa_{44} is Pro, amide or is absent;
Xaa_{45} is Ser, amide or is absent;
Xaa_{46} is amide or is absent;

provided that if Xaa_{38}, Xaa_{39}, Xaa_{40}, Xaa_{41}, Xaa_{42}, Xaa_{43}, Xaa_{44}, Xaa_{45} or Xaa_{46} is absent then each amino acid residue downstream is also absent.

In one embodiment the GLP-1 peptide comprises the amino acid sequence of formula (II) (SEQ ID NO: 6):

Formula (II)
Xaa_7-Xaa_8-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-

Xaa_{18}-Tyr-Leu-Glu-Xaa22-Xaa_{23}-Ala-Ala-Xaa_{26}-Glu-

Phe-Ile-Xaa_{30}-Trp-Leu-Val-Xaa_{34}-Xaa_{35}-Xaa_{36}-

$Xaa_{37}Xaa_{38}$

wherein

Xaa_7 is L-histidine, D-histidine, desamino-histidine, 2-amino-histidine, -hydroxy-histidine, homohistidine, N^{α}-acetyl-histidine, α-fluoromethyl-histidine, α-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine;
Xaa_8 is Ala, Gly, Val, Leu, Ile, Lys, Aib, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid;
Xaa_{18} is Ser, Lys or Arg;
Xaa_{22} is Gly, Glu or Aib;
Xaa_{23} is Gln, Glu, Lys or Arg;
Xaa_{26} is Lys, Glu or Arg; Xaa30 is Ala, Glu or Arg;
Xaa_{34} is Lys, Glu or Arg;
Xaa_{35} is Gly or Aib;
Xaa_{36} is Arg or Lys;
Xaa_{37} is Gly, Ala, Glu or Lys;
Xaa_{38} is Lys, amide or is absent.

In one embodiment the GLP-1 peptide is a DPPIV protected GLP-1 peptide.

In one embodiment the GLP-1 peptide is DPPIV stabilised.

In one embodiment the GLP-1 peptide comprises an Aib residue in position 8.

In one embodiment the amino acid residue in position 7 of said GLP-1 peptide is selected from the group consisting of D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, N^{α}-acetyl-histidine, α-fluoromethyl-histidine, α-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine and 4-pyridylalanine.

In one embodiment the GLP-1 peptide comprises Arg^{34}GLP-1 (7-37) or [Aib8, Arg34]G LP-1-(7-37).

In one embodiment the GLP-1 agonist comprises an albumin binding residue which is covalently attached, optionally via a hydrophilic spacer. In one embodiment said albumin binding residue is covalently attached, optionally via a hydrophilic spacer, to the C-terminal amino acid residue of said GLP-1 peptide or an amino acid residue which is not the C-terminal amino acid residue. In one embodiment the GLP-1 peptide is attached to a hydrophilic spacer via the amino acid residue in position 23, 26, 34, 36 or 38 relative to the amino acid sequence of GLP-1 (7-37).

Human Glucagon-Like Peptide-1 is GLP-1 (7-37) and has the sequence HAEGTFTSDVSSYLEGQAAKEFI AWLVKGRG (SEQ ID NO: 1). GLP-1(7-37) may also be designated "native" GLP-1. In the sequence listing, the first amino acid residue of SEQ ID NO: 1 (histidine) is assigned no. 1. However, in what follows—according to established practice in the art—this histidine residue is referred to as no. 7, and subsequent amino acid residues are numbered accordingly, ending with glycine no. 37. Therefore, generally, any reference herein to an amino acid residue number or a position number of the GLP-1(7-37) sequence is to the sequence starting with His at position 7 and ending with Gly at position 37. A non-limiting example of a suitable analogue nomenclature is [Aib^8, Arg^{34}, Lys^{37}]GLP-1(7-37), which designates a GLP-1(7-37) analogue, in which the alanine at position 8 has been substituted with α-aminoisobutyric acid (Aib), the lysine at position 34 has been substituted with arginine, and the glycine at position 37 has been substituted with lysine.

In one embodiment the GLP-1 agonist exhibits at least 60%, 65%, 70%, 80% or 90% sequence identity to GLP-1 (7-37) over the entire length of GLP-1(7-37). As an example of a method for determination of sequence identity between two analogues the two peptides [Aib8]GLP-1(7-37) and GLP-1(7-37) are aligned. The sequence identity of [Aib8] GLP-1(7-37) relative to GLP-1(7-37) is given by the number of aligned identical residues minus the number of different residues divided by the total number of residues in GLP-1 (7-37). Accordingly, in said example the sequence identity is (31-1)/31. In one embodiment non-peptide moieties of the GLP-1 agonist are not included when determining sequence identity.

In one embodiment the GLP-1 agonist is a derivative. In one embodiment the term "derivative" as used herein in the context of a GLP-1 agonist, peptide or analogue means a chemically modified GLP-1 agonist, peptide or analogue, in which one or more substituents have been covalently attached to the agonist, peptide or analogue. The substituent may also be referred to as a side chain. Typical modifications are amides, carbohydrates, alkyl groups, acyl groups, esters and the like. An example of a derivative of GLP-1(7-37) is $N^{\varepsilon 26}$-(γ-Glu(N^{α}-hexadecanoyl))-[Arg^{34}, Lys^{25}]) GLP-1 (7-37).

In a particular embodiment, the side chain is capable of forming non-covalent aggregates with albumin, thereby promoting the circulation of the GLP-1 agonist with the blood stream, and also having the effect of protracting the time of action of the GLP-1 agonist, due to the fact that the aggregate of the GLP-1 agonist and albumin is only slowly disintegrated to release the active pharmaceutical ingredient. Thus, the substituent, or side chain, as a whole may be referred to as an albumin binding moiety.

In particular embodiments, the side chain has at least 10 carbon atoms, or at least 15, 20, 25, 30, 35, or at least 40 carbon atoms. In further particular embodiments, the side chain may further include at least 5 hetero atoms, in particular 0 and N, for example at least 7, 9, 10, 12, 15, 17, or at least 20 hetero atoms, such as at least 1, 2, or 3 N-atoms, and/or at least 3, 6, 9, 12, or 15 O-atoms.

In another particular embodiment the albumin binding moiety comprises a portion which is particularly relevant for the albumin binding and thereby the protraction, which portion may accordingly be referred to as a protracting moiety. The protracting moiety may be at, or near, the opposite end of the albumin binding moiety, relative to its point of attachment to the peptide.

In a still further particular embodiment the albumin binding moiety comprises a portion in between the protracting moiety and the point of attachment to the peptide, which portion may be referred to as a linker, linker moiety, spacer, or the like. The linker may be optional, and hence in that case the albumin binding moiety may be identical to the protracting moiety.

In particular embodiments, the albumin binding moiety and/or the protracting moiety is lipophilic, and/or negatively charged at physiological pH (7.4).

The albumin binding moiety, the protracting moiety, or the linker may be covalently attached to a lysine residue of the GLP-1 peptide by acylation. Additional or alternative conjugation chemistry includes alkylation, ester formation, or amide formation, or coupling to a cysteine residue, such as by maleimide or haloacetamide (such as bromo-/fluoro-/iodo-) coupling.

In one embodiment an active ester of the albumin binding moiety, e.g. comprising a protracting moiety and a linker, is covalently linked to an amino group of a lysine residue, e.g. the epsilon amino group thereof, under formation of an amide bond (this process being referred to as acylation).

Unless otherwise stated, when reference is made to an acylation of a lysine residue, it is understood to be to the epsilon-amino group thereof.

For the present purposes, the terms "albumin binding moiety", "protracting moiety", and "linker" may include the unreacted as well as the reacted forms of these molecules. Whether or not one or the other form is meant is clear from the context in which the term is used.

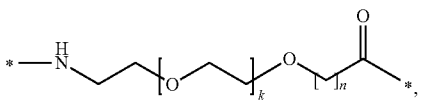
For the attachment to the GLP-1 agonist, the acid group of the fatty acid, or one of the acid groups of the fatty diacid, forms an amide bond with the epsilon amino group of a lysine residue in the GLP-1 peptide, e.g. via a linker.

In one embodiment the term "fatty acid" refers to aliphatic monocarboxylic acids having from 4 to 28 carbon atoms, it is optionally unbranched, and/or even numbered, and it may be saturated or unsaturated.

In one embodiment the term "fatty diacid" refers to fatty acids as defined above but with an additional carboxylic acid group in the omega position. Thus, fatty diacids are dicarboxylic acids.

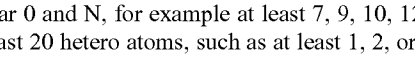
Each of the two linkers of the GLP-1 agonist of the invention may comprise the following first linker element:

Chem. 5



wherein k is an integer in the range of 1-5, and n is an integer in the range of 1-5.

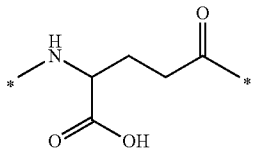
In a particular embodiment, when k=1 and n=1, this linker element may be designated OEG, or a di-radical of 8-amino-3,6-dioxaoctanic acid, and/or it may be represented by the following formula:



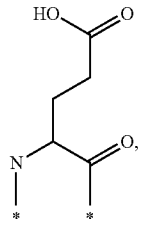
Chem. 5a:

In another particular embodiment, each linker of the GLP-1 agonist of the invention may further comprise, independently, a second linker element, e.g. a Glu di-radical, such as Chem. 6 and/or Chem. 7:

Chem. 6



Chem. 7



wherein the Glu di-radical may be included p times, where p is an integer in the range of 1-3.

Chem. 6 may also be referred to as gamma-Glu, or briefly gGlu, due to the fact that it is the gamma carboxy group of the amino acid glutamic acid which is here used for connection to another linker element, or to the epsilon-amino group of lysine. As explained above, the other linker element may, for example, be another Glu residue, or an OEG molecule. The amino group of Glu in turn forms an amide bond with the carboxy group of the protracting moiety, or with the carboxy group of, e.g., an OEG molecule, if present, or with the gamma-carboxy group of, e.g., another Glu, if present.

Chem. 7 may also be referred to as alpha-Glu, or briefly aGlu, or simply Glu, due to the fact that it is the alpha carboxy group of the amino acid glutamic acid which is here used for connection to another linker element, or to the epsilon-amino group of lysine.

The above structures of Chem. 6 and Chem. 7 cover the L-form, as well as the D-form of Glu. In particular embodiments, Chem. 6 and/or Chem. 7 is/are, independently, a) in the L-form, or b) in the D-form.

In still further particular embodiments the linker has a) from 5 to 41 C-atoms; and/or b) from 4 to 28 hetero atoms.

The concentration in plasma of the GLP-1 agonists of the invention may be determined using any suitable method. For example, LC-MS (Liquid Chromatography Mass Spectroscopy) may be used, or immunoassays such as RIA (Radio Immuno Assay), ELISA (Enzyme-Linked Immuno Sorbent Assay), and LOCI (Luminescence Oxygen Channeling Immunoassay). General protocols for suitable RIA and ELISA assays are found in, e.g., WO09/030738 on p. 116-118. A preferred assay is the LOCI (Luminescent Oxygen Channeling Immunoassay), generally as described for the determination of insulin by Poulsen and Jensen in Journal of Biomolecular Screening 2007, vol. 12, p. 240-247—briefly blood samples may be collected at desired intervals, plasma separated, immediately frozen, and kept at

−20° C. until analyzed for plasma concentration of the respective GLP-1 agonist; the donor beads are coated with streptavidin, while acceptor beads are conjugated with a monoclonal antibody recognising a mid-/C-terminal epitope of the peptide; another monoclonal antibody, specific for the N-terminus, is biotinylated; the three reactants are combined with the analyte and formed a two-sited immuno-complex; illumination of the complex releases singlet oxygen atoms from the donor beads, which are channeled into the acceptor beads and triggered chemiluminescence which may be measured in an Envision plate reader; the amount of light is proportional to the concentration of the compound.

In one embodiment the term "Aib" as used herein refers to α-aminoisobutyric acid.

Pharmaceutical Compositions

An administered dose may contain from 5 mg-100 mg of the GLP-1 agonist, or from 5-50 mg, or from 5-20 mg, or from 5-10 mg of the GLP-1 agonist.

In one embodiment the composition is BYDUREON® (a long acting release formula of exenatide in PLGA particles).

Pharmaceutical compositions comprising a GLP-1 agonist of the invention or a pharmaceutically acceptable salt, amide, or ester thereof, and a pharmaceutically acceptable excipient may be prepared as is known in the art.

In one embodiment the term "excipient" broadly refers to any component other than the active therapeutic ingredient(s). The excipient may be an inert substance, an inactive substance, and/or a not medicinally active substance. The formulation of pharmaceutically active ingredients with various excipients is known in the art, see e.g. Remington: The Science and Practice of Pharmacy (e.g. 19th edition (1995), and any later editions). Non-limiting examples of excipients are: solvents, diluents, buffers, preservatives, tonicity regulating agents (e.g isotonic agents), chelating agents, stabilisers (e.g. oxidation inhibitors, aggregation inhibitors, surfactants, and/or protease inhibitors).

Examples of formulations include liquid formulations, i.e. aqueous formulations comprising water. A liquid formulation may be a solution, or a suspension. An aqueous formulation typically comprises at least 50% w/w water, or at least 60%, 70%, 80%, or even at least 90% w/w of water.

Alternatively, a pharmaceutical composition may be a solid formulation, e.g. a freeze-dried or spray-dried composition, which may be used as is, or whereto the physician or the patient adds solvents, and/or diluents prior to use.

The pH in an aqueous formulation may be anything between pH 3 and pH 10, for example from about 7.0 to about 9.5; or from about 3.0 to about 7.0.

Still further, a pharmaceutical composition may be formulated as is known in the art of oral formulations of insulinotropic compounds, e.g. using any one or more of the formulations described in WO 2008/145728.

A composition may be administered in several dosage forms, for example as a solution; a suspension; an emulsion; a microemulsion; multiple emulsions; a foam; a salve; a paste; a plaster; an ointment; a tablet; a coated tablet; a chewing gum; a rinse; a capsule such as hard or soft gelatine capsules; a suppositorium; a rectal capsule; drops; a gel; a spray; a powder; an aerosol; an inhalant; eye drops; an ophthalmic ointment; an ophthalmic rinse; a vaginal pessary; a vaginal ring; a vaginal ointment; an injection solution; an in situ transforming solution such as in situ gelling, setting, precipitating, and in situ crystallisation; an infusion solution; or as an implant.

A composition may further be compounded in a drug carrier or drug delivery system, e.g. in order to improve stability, bioavailability, and/or solubility. In a particular embodiment a composition may be attached to such system through covalent, hydrophobic, and/or electrostatic interactions. The purpose of such compounding may be, e.g., to decrease adverse effects, achieve chronotherapy, and/or increase patient compliance.

A composition may also be used in the formulation of controlled, sustained, protracting, retarded, and/or slow release drug delivery systems.

The composition may be administered by parenteral administration. Parenteral administration may be performed by subcutaneous, intramuscular, intraperitoneal, or intravenous injection by means of a syringe, optionally a pen-like syringe, or by means of an infusion pump.

Production Processes

In one embodiment GLP-1 peptides can be produced by appropriate derivatisation of an appropriate peptide backbone which has been produced by recombinant DNA technology or by peptide synthesis (e.g., Merrifield-type solid phase synthesis) as known in the art of peptide synthesis and peptide chemistry.

In one embodiment the production of peptides like GLP-1(7-37) and GLP-1 analogues is well known in the art. The GLP-1 moiety of the GLP-1 peptide of the invention (or fragments thereof) may for instance be produced by classical peptide synthesis, e.g., solid phase peptide synthesis using t-Boc or Fmoc chemistry or other well established techniques, see, e.g., Greene and Wuts, "Protective Groups in Organic Synthesis", John Wiley & Sons, 1999, Florencio Zaragoza Dörwald, "Organic Synthesis on solid Phase", Wiley-VCH Verlag GmbH, 2000, and "Fmoc Solid Phase Peptide Synthesis", Edited by W. C. Chan and P. D. White, Oxford University Press, 2000.

In one embodiment GLP-1 agonists may be produced by recombinant methods, viz. by culturing a host cell containing a DNA sequence encoding the GLP-1 agonist and capable of expressing the peptide in a suitable nutrient medium under conditions permitting the expression of the peptide. Non-limiting examples of host cells suitable for expression of these peptides are: *Escherichia coli, Saccharomyces cerevisiae*, as well as mammalian BHK or CHO cell lines.

In one embodiment GLP-1 agonists of the invention which include non-natural amino acids and/or a covalently attached N-terminal mono- or dipeptide mimetic may e.g. be produced as described in the experimental part. Or see e.g., Hodgson et al: "The synthesis of peptides and proteins containing non-natural amino acids", Chemical Society Reviews, vol. 33, no. 7 (2004), p. 422-430; and WO 2009/083549 Al entitled "Semi-recombinant preparation of GLP-1 analogues".

EMBODIMENTS

The following are non-limiting embodiments of the invention:

1. A method for reduction of HbA1c or for prevention or treatment of type 2 diabetes, hyperglycemia, impaired glucose tolerance, or non-insulin dependent diabetes, said method comprising administration of a GLP-1 agonist to a subject in need thereof in an amount of at least 0.7 mg per week, such an amount equivalent to at least 0.7 mg semaglutide per week.

2. A method for treating or preventing obesity, for reducing body weight and/or food intake, or for inducing satiety, said method comprising administration of a GLP-1 agonist to a

subject in need thereof in an amount of at least 0.7 mg per week, such an amount equivalent to at least 0.7 mg semaglutide per week.

3. The method according to any one of the preceding embodiments, wherein said method comprises delaying or preventing diabetic disease progression.

4. The method according to any one of the preceding embodiments, wherein said GLP-1 agonist has a half-life of at least 24 hours, such as at least 48 hours, at least 60 hours, or at least 72 hours, or such as at least 84 hours, at least 96 hours, or at least 108 hours, or optionally at least 120 hours, at least 132 hours, or at least 144 hours, wherein said half-life optionally is determined by Assay (II).

5. The method according to any one of the preceding embodiments, wherein said GLP-1 agonist is administered twice weekly or less often, once weekly or less often, such as less often than once weekly or once every secondly week or less often, or such as once every third week or less often or once a month or less often.

6. The method according to any one of the preceding embodiments, wherein said GLP-1 agonist is administered in an amount of at least 0.8 mg, at least 1.0 mg, or at least 1.2 mg, such as at least 1.4 mg or at least 1.6 mg, per week.

7. The method according to any one of the preceding embodiments, wherein said GLP-1 agonist is administered in an amount equivalent to at least 0.8 mg, at least 1.0 mg, or at least 1.2 mg, such as at least 1.4 mg or at least 1.6 mg, semaglutide per week.

8. The method according to any one of the preceding embodiments, wherein said GLP-1 agonist is administered in an amount which provides an improved a) reduction in HbA1c or b) reduction in body weight compared to administration of 1.8 mg liraglutide or less, such as 0.8 mg liraglutide or less, per day.

9. The method according to any one of the preceding embodiments, wherein said GLP-1 agonist is selected from the group consisting of semaglutide, exenatide, albiglutide, and dulaglutide.

10. The method according to any one of the preceding embodiments, wherein said GLP-1 agonist is administered by parenteral administration, such as subcutaneous injection.

11. The method according to any one of the preceding embodiments, wherein said GLP-1 agonist is administered simultaneously or sequentially with another therapeutic agent.

12. The method according to any one of any one of the preceding embodiments, wherein the GLP-1 agonist is a GLP-1 peptide.

13. The method according to any one of the preceding embodiments, wherein the GLP-1 peptide comprises the amino acid sequence of the formula (I) (SEQ ID NO: 5):

Formula (I)
Xaa_7-Xaa_8-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Xaa_{16}-Ser-
Xaa_{18}-$Xaa_{19}$$Xaa_{20}$GluXaa$_{22}$-$Xaa_{23}$-Ala-$Xaa_{25}$-$Xaa_{26}$-$Xaa_{27}$-
Phe-Ile-Xaa_{30}-Trp-Leu-Xaa_{33}-Xaa_{34}-Xaa_{35}-Xaa_{36}-Xaa_{37}-
Xaa_{38}-Xaa_{39}-Xaa_{40}-Xaa_{41}-Xaa_{42}-Xaa_{43}-Xaa_{44}-Xaa_{45}-Xaa_{46}

wherein

Xaa_7 is L-histidine, D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, N^{α}-acetyl-histidine, α-fluoromethyl-histidine, α-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine;

Xaa_8 is Ala, Gly, Val, Leu, Ile, Lys, Aib, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid;

Xaa_{16} is Val or Leu;

Xaa_{18} is Ser, Lys or Arg;

Xaa_{19} is Tyr or Gln;

Xaa_{20} is Leu or Met;

Xaa_{22} is Gly, Glu or Aib;

Xaa_{23} is Gln, Glu, Lys or Arg;

Xaa_{25} is Ala or Val;

Xaa_{26} is Lys, Glu or Arg;

Xaa_{27} is Glu or Leu;

Xaa_{30} is Ala, Glu or Arg;

Xaa_{33} is Val or Lys;

Xaa_{34} is Lys, Glu, Asn or Arg;

Xaa_{35} is Gly or Aib;

Xaa_{36} is Arg, Gly or Lys;

Xaa_{37} is Gly, Ala, Glu, Pro, Lys, amide or is absent;

Xaa_{38} is Lys, Ser, amide or is absent;

Xaa_{39} is Ser, Lys, amide or is absent;

Xaa_{40} is Gly, amide or is absent;

Xaa_{41} is Ala, amide or is absent;

Xaa_{42} is Pro, amide or is absent;

Xaa_{43} is Pro, amide or is absent;

Xaa_{44} is Pro, amide or is absent;

Xaa_{45} is Ser, amide or is absent;

Xaa_{46} is amide or is absent;

provided that if Xaa_{38}, Xaa_{39}, Xaa_{40}, Xaa_{41}, Xaa_{42}, Xaa_{43}, Xaa_{44}, Xaa_{45} or Xaa_{46} is absent then each amino acid residue downstream is also absent.

14. The method according to any one of the preceding embodiments, wherein said polypeptide is a GLP-1 peptide comprising the amino acid sequence of formula (II) (SEQ ID NO: 6):

Formula (II)
Xaa_7-Xaa_8-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-
Xaa_{18}-Tyr-Leu-Glu-Xaa22-Xaa_{23}-Ala-Ala-Xaa_{26}-Glu-
Phe-Ile-Xaa_{30}-Trp-Leu-Val-Xaa_{34}-Xaa_{35}-Xaa_{36}-
$Xaa_{37}$$Xaa_{38}$

wherein

Xaa_7 is L-histidine, D-histidine, desamino-histidine, 2-amino-histidine, -hydroxy-histidine, homohistidine, N^{α}-acetyl-histidine, α-fluoromethyl-histidine, α-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine;

Xaa_8 is Ala, Gly, Val, Leu, Ile, Lys, Aib, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid;

Xaa_{18} is Ser, Lys or Arg;

Xaa_{22} is Gly, Glu or Aib;

Xaa_{23} is Gln, Glu, Lys or Arg;

Xaa_{26} is Lys, Glu or Arg; Xaa30 is Ala, Glu or Arg;

Xaa_{34} is Lys, Glu or Arg;

Xaa_{35} is Gly or Aib;

Xaa_{36} is Arg or Lys;

Xaa_{37} is Gly, Ala, Glu or Lys;

Xaa_{38} is Lys, amide or is absent.

15. The method according to any one of the preceding embodiments, wherein said GLP-1 peptide is selected from GLP-1 (7-35), GLP-1 (7-36), GLP-1 (7-36)-amide, GLP-1

(7-37), GLP-1 (7-38), GLP-1 (7-39), GLP-1 (7-40), GLP-1 (7-41) or an analogue or derivative thereof.

16. The method according to any one of the preceding embodiments, wherein said GLP-1 peptide comprises no more than 15, such as no more than 10 or no more than 6, amino acid residues which have been substituted, inserted or deleted as compared to GLP-1 (7-37).

17. The method according to any one of the preceding embodiments, wherein said GLP-1 peptide comprises no more than 5 amino acid residues which have been substituted, inserted or deleted as compared to GLP-1 (7-37).

18. The method according to any one of the preceding embodiments, wherein said GLP-1 peptide comprises no more than 4 amino acid residues which are not encoded by the genetic code.

19. The method according to any one of the preceding embodiments, wherein said GLP-1 peptide is a DPPIV protected GLP-1 peptide.

20. The method according to any one of the preceding embodiments, wherein GLP-1 peptide is DPPIV stabilised.

21. The method according to any one of the preceding embodiments, wherein said GLP-1 peptide comprises an Aib residue in position 8.

22. The method according to any one of the preceding embodiments, wherein the amino acid residue in position 7 of said GLP-1 peptide is selected from the group consisting of D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, N^{α}-acetyl-histidine, α-fluoromethyl-histidine, α-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine and 4-pyridylalanine.

23. The method according to any one of embodiments 7 to 16, wherein said GLP-1 peptide is attached to said hydrophilic spacer via the amino acid residue in position 23, 26, 34, 36 or 38 relative to the amino acid sequence of GLP-1 (7-37).

24. The method according to any one of the preceding embodiments, wherein the GLP-1 peptide is exendin-4, an exendin-4-analogue, or a derivative of exendin-4.

25. The method according to any one of the preceding embodiments, wherein the GLP-1 peptide comprises the amino acid sequence of the following formula:

H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH_2 (SEQ ID NO: 2).

26. The method according to any one of the preceding embodiments wherein one albumin binding residue via said hydrophilic spacer is attached to the C-terminal amino acid residue of said GLP-1 peptide.

27. The method according to any one of the preceding embodiments, wherein a second albumin binding residue is attached to an amino acid residue which is not the C-terminal amino acid residue.

28. The method according to any one of the preceding embodiments, wherein the GLP-1 peptide is selected from the group consisting of semaglutide, albiglutide and dulaglitide.

29. The method according to any one of the preceding embodiments, wherein the GLP-1 peptide has the following structure:

(SEQ ID NO: 3)
His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Aib-Arg.

30. The method according to any one of the preceding embodiments, wherein the GLP-1 peptide has the following structure:

(SEQ ID NO: 4)
(His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg)2

genetically fused to human albumin.

31. The method according to any one of the preceding embodiments wherein the GLP-1 peptide is dulaglitide.

32. The method according to any one of the preceding embodiments wherein the GLP-1 agonist has an EC_{50} at or below 3000 pM, such as at or below 500 pM or at or below 100 pM, optionally determined by Assay (I).

33. A GLP-1 agonist for use in the reduction of HbA1c or for use in the prevention or treatment of type 2 diabetes, hyperglycemia, impaired glucose tolerance, or non-insulin dependent diabetes comprising administering a GLP-1 agonist in an amount of at least 0.7 mg per week, such an amount equivalent to at least 0.7 mg semaglutide per week.

34. A GLP-1 agonist for use in the prevention or treatment of obesity, in the reduction of body weight and/or food intake, or in the induction satiety comprising administering a GLP-1 agonist in an amount of at least 0.7 mg per week, such an amount equivalent to at least 0.7 mg semaglutide per week.

35. A GLP-1 agonist for use according to embodiment 33 or 34, wherein the GLP-1 agonist and/or administration is as defined in any of embodiments 1-32 or 40.

36. A composition comprising a GLP-1 agonist and one or more pharmaceutically acceptable excipients for use in reduction of HbA1c or for prevention or treatment of type 2 diabetes, hyperglycemia, impaired glucose tolerance, or non-insulin dependent diabetes, wherein said GLP-1 agonist is administered in an amount of at least 0.7 mg per week, such an amount equivalent to at least 0.7 mg semaglutide per week.

37. A composition comprising a GLP-1 agonist and one or more pharmaceutically acceptable excipients for use in the prevention or treatment of obesity, in the reduction of body weight and/or food intake, or in the induction satiety, wherein said GLP-1 agonist is administered in an amount of at least 0.7 mg per week, such an amount equivalent to at least 0.7 mg semaglutide per week.

38. A composition for use according to any one of the preceding embodiments, wherein said GLP-1 agonist and/or administration is as defined in any one of embodiments 1-32 or 40.

39. A composition for use according to any one of the preceding embodiments, wherein said composition comprises the Bydureon® composition.

40. The method according to any one of the preceding claims, wherein the method comprises prevention, treatment, reduction or induction in one or more diseases or conditions defined in any one of the previous embodiments.

EXAMPLES

Abbreviations

The following abbreviations are used in the following, in alphabetical order:

ADA: American Diabetes Association

Example 1: The Glp-1 Peptide Semaglutide Provides Reduced HbA1c and Body Weight

Semaglutide is a unique acylated GLP-1 peptide with a half-life of 160 hours. The aim was to investigate HbA1c

dose-response of once-weekly doses of semaglutide (five dose-levels) in subjects with type 2 diabetes. Safety, tolerability and pharmacodynamics of semaglutide versus placebo and open-label once-daily liraglutide were also investigated.

Materials and Methods

Liraglutide may be prepared as described in Example 37 of WO98/08871. Semaglutide may be prepared as described in Example 4 of WO2006/097537. The composition of the GLP-1 agonists administered may be formulated as isotonic aqueous solutions with a phosphate buffer, such as a sodium dihydrogen phosphate buffer, having a pH in the range 7.0-9.0, such as pH 7.4 or pH 8.15, for example further comprising the excipients propylene glycol and phenol. The composition of the GLP-1 agonists administered may be as described in WO2003/002136 or WO2005/049061. The placebo composition may be identical to the composition of the GLP-1 agonists, but not containing a GLP-1 agonist.

In a 12-week, randomised, double-blind, placebo-controlled trial, 411 human subjects (n=43-50 per group) with type 2 diabetes were exposed. Participants (male/female 65/35%; baseline HbA1c (mean±SD) 8.1±0.8%; baseline body weight 87.5±13.8 kg; duration of diabetes 2.6±3.1 years; metformin only/diet and exercise alone 80/20%) received subcutaneous injection of one of five semaglutide doses (0.1-1.6 mg) once weekly, open-label liraglutide (1.2 mg, 1.8 mg) once daily, or placebo once weekly. Two of the semaglutide doses were titrated (T) in weekly increments of 0.4 mg. The primary endpoint was change in HbA1c from baseline. Secondary efficacy endpoints included proportion of subjects reaching ADA HbA1c target (<7%) and change in body weight. Change and percentage to target were analysed by ANOVA and logistic regression, respectively. Comparisons between semaglutide and liraglutide were not corrected for multiplicity. Baseline characteristics of the subjects are shown in Table 1.

Results

In the full analysis set, semaglutide (≥0.2 mg) dose-dependently reduced HbA1c from baseline (FIG. **1**), and increased the likelihood of achieving HbA1c<7% ($p<0.05$ vs. placebo for doses ≥0.2 mg). The results with respect to change in HbA1c are shown in FIG. **1**. The change in HbA1c in FIG. **1** is from baseline at week 12. FIG. **2** shows the change in HbA1c over time with the different treatments. Treatment with semaglutide 13.8 mg numerically brought more patients to target than liraglutide 1.8 mg (0.8 mg T 69%, 0.8 mg 73%, 1.6 mg T 81% vs. liraglutide 1.8 mg 57%).The results (see e.g. FIG. **1**) shows that treatment with semaglutide 0.8 mg, 0.8 mg T, or 1.6 mg T improved reduction of HbA1c compared to treatment with liraglutide 1.2 mg or 1.8 mg; furthermore, treatment with semaglutide 1.6 mg T was statistically superior to treatment with liraglutide 1.2 mg or 1.8 mg with respect to reduction of HbA1c (based on unadjusted means). FIG. **3** shows the percentage and the number of subjects reaching the AACE or ADA criteria for glycaemic control with the different treatments. The results (see FIG. **3**) shows that treatment with semaglutide 0.8 mg, 0.8 mg T, or 1.6 mg T improved the percentage and the number of subjects reaching the AACE or ADA criteria for glycaemic control compared to treatment with liraglutide 1.2 mg or 1.8 mg.

Body weight was dose-dependently reduced from baseline by up to 4.8 kg vs. placebo 1.2 kg ($p<0.01$ for doses 13.8 mg). FIGS. **4** and **5** shows mean body weight change versus time and body weight change from baseline at week 12, respectively, with the different treatments. The results (see e.g. FIG. **5**) shows that treatment with semaglutide 0.8 mg, 0.8 mg T, or 1.6 mg T increased reduction of body weight compared to treatment with liraglutide 1.2 mg or 1.8 mg. Furthermore, the results (see e.g. FIG. **5**) shows that treatment with semaglutide 0.8 mg T or 1.6 mg T was statistically superior to treatment with liraglutide 1.8 mg with respect to reduction of body weight; and that treatment with semaglutide 0.8 mg, 0.8 mg T, or 1.6 mg T was statistically

TABLE 1

Baseline characteristics of subjects

		Semaglutide						Liraglutide	
	Placebo	0.1 mg	0.2 mg	0.4 mg	0.8 mg	0.8 mg T	1.6 mg T	1.2 mg	1.8 mg
Exposed* subjects	46	47	43	48	42	43	47	45	50
D&E: metformin (%)	22:78	23:77	14:86	23:77	19:81	16:84	19:81	18:82	24:76
Female: male (%)	39:61	34:66	30:70	23:77	48:52	37:63	45:55	31:69	30:70
Age (years)	55.3 (10.6)	55.2 (10.1)	54.7 (10.0)	53.8 (10.2)	55.0 (9.7)	55.9 (7.9)	56.4 (10.5)	54.8 (9.2)	54.3 (10.1)
Duration of diabetes (years)	2.4 (3.3)	3.6 (5.0)	2.3 (2.7)	2.0 (2.3)	3.0 (3.0)	2.6 (2.1)	1.8 (2.0)	3.3 (3.4)	2.5 (2.6)
HbA1c (%)	8.1 (0.8)	8.2 (0.9)	8.2 (0.9)	8.1 (0.9)	8.2 (0.9)	8.0 (0.8)	8.0 (0.7)	8.0 (0.8)	8.1 (0.7)
FPG (mmol/L)	8.9 (1.5)	9.8 (2.7)	9.5 (2.5)	9.3 (2.1)	9.5 (2.4)	9.6 (2.1)	9.0 (1.9)	9.0 (2.3)	9.3 (2.0)
Weight (kg)	90.5 (13.0)	89.5 (14.2)	86.3 (15.1)	87.0 (14.0)	85.9 (15.1)	85.7 (12.6)	84.5 (14.0)	90.5 (13.5)	87.2 (13.1)
BMI (kg/m^2)	31.7 (3.8)	31.5 (4.6)	30.4 (3.9)	29.7 (4.5)	30.7 (4.5)	31.2 (4.2)	30.9 (4.7)	31.0 (4.6)	30.9 (4.6)

Data are mean (SD) unless otherwise stated.

*Number of subjects exposed to actual treatment. D&E: Diet and exercise. FPG: Fasting plasma glucose. BMI: Body mass index.

superior to liraglutide 1.2 mg with respect to reduction of body weight (based on unadjusted means).

There were no reports of pancreatitis or treatment-related changes in blood calcitonin. Proportion of subjects with nausea and vomiting increased with dose, but were generally mild or moderate and ameliorated by titration. Withdrawals due to gastrointestinal adverse events were 4.7%-27.7% for semaglutide and 2.2%-10% for liraglutide. Few subjects reported minor hypoglycaemia (semaglutide n=5, liraglutide n=3); no major hypoglycaemia. Injection site reactions were reported by 7 subjects: semaglutide n=2; liraglutide n=5. One subject (semaglutide 1.6 mg T) developed low titre non-neutralising anti-semaglutide antibodies (no cross-reaction to native GLP-1).

CONCLUSION

Over 12 weeks, semaglutide dose-dependently reduced HbA1c and body weight. The effect of semaglutide 0.4 mg on glycaemic control and body weight was comparable to that of liraglutide 1.2 mg, while semaglutide ≥0.8 mg appeared to bring more subjects to target and provided better weight loss than liraglutide 1.8 mg. No semaglutide safety concerns were identified. Dose escalation was not a major focus of this trial and it will be optimised in future clinical trials.

Pharmacological Methods

Assay (I): In Vitro Potency

The purpose of this example is to test the activity, or potency, of GLP-1 agonists in vitro. The potencies of GLP-1 agonists may be determined as described below, i.e. as the stimulation of the formation of cyclic AMP (cAMP) in a medium containing membranes expressing the human GLP-1 receptor.

Principle

Purified plasma membranes from a stable transfected cell line, BHK467-12A (tk-ts13), expressing the human GLP-1 receptor are stimulated with the GLP-1 agonist in question, and the potency of cAMP production is measured using the AlphaScreen™ cAMP Assay Kit from Perkin Elmer Life Sciences. The basic principle of The AlphaScreen Assay is a competition between endogenous cAMP and exogenously added biotin-cAMP. The capture of cAMP is achieved by using a specific antibody conjugated to acceptor beads.

Cell Culture and Preparation of Membranes

A stable transfected cell line and a high expressing clone are selected for screening. The cells are grown at 5% CO_2 in DMEM, 5% FCS, 1% Pen/Strep (Penicillin/Streptomycin) and 0.5 mg/ml of the selection marker G418.

Cells at approximate 80% confluence are washed 2× with PBS and harvested with Versene (aqueous solution of the tetrasodium salt of ethylenediaminetetraacetic acid), centrifuged 5 min at 1000 rpm and the supernatant removed. The additional steps are all made on ice. The cell pellet is homogenised by the Ultrathurax for 20-30 sec. in 10 ml of Buffer 1 (20 mM Na-HEPES, 10 mM EDTA, pH=7.4), centrifuged 15 min at 20,000 rpm and the pellet resuspended in 10 ml of Buffer 2 (20 mM Na-HEPES, 0.1 mM EDTA, pH=7.4). The suspension is homogenised for 20-30 sec and centrifuged 15 min at 20,000 rpm. Suspension in Buffer 2, homogenisation and centrifugation is repeated once and the membranes are resuspended in Buffer 2. The protein concentration is determined and the membranes stored at −80° C. until use.

The assay is performed in ½-area 96-well plates, flat bottom (e.g. Costar cat. no: 3693).

The final volume per well is 50 μl.

Solutions and Reagents

Exemplary solutions and reagents are given below.

AlphaScreen cAMP Assay Kit from Perkin Elmer Life Sciences (cat. No: 6760625M); containing Anti-cAMP Acceptor beads (10 U/μl), Streptavidin Donor beads (10 U/μl) and Biotinylated-cAMP (133 U/μl).

AlphaScreen Buffer, pH=7.4: 50 mM TRIS-HCl (Sigma, cat. no: T3253); 5 mM HEPES (Sigma, cat. no: H3375); 10 mM $MgCl_2$, $6H_2O$ (Merck, cat. no: 5833); 150 mM NaCl (Sigma, cat. no: S9625); 0.01% Tween (Merck, cat. no: 822184). The following was added to the AlphaScreen Buffer prior to use (final concentrations indicated): BSA (Sigma, cat. no. A7906): 0.1%; IBMX (Sigma, cat. no. 15879): 0.5 mM; ATP (Sigma, cat. no. A7699): 1 mM; GTP (Sigma, cat. no. G8877): 1 μM.

cAMP standard (dilution factor in assay=5): cAMP Solution: 5 μL of a 5 mM cAMP-stock+495 μL AlphaScreen Buffer.

Suitable dilution series in AlphaScreen Buffer are prepared of the cAMP standard as well as the GLP-1 agonist to be tested, e.g. the following eight concentrations of the GLP-1 agonist: 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11}, 10^{-12}, 10^{-13} and 10^{-14}M, and a series from, e.g., 10^{-6} to 3×10^{-11} of cAMP.

Membrane/Acceptor Beads

Use hGLP-1/BHK 467-12A membranes; 6 μg/well corresponding to 0.6 mg/ml (the amount of membranes used pr. well may vary)

"No membranes": Acceptor Beads (15 μg/ml final) in AlphaScreen buffer

"6 μg/well membranes": membranes+Acceptor Beads (15 μg/ml final) in AlphaScreen buffer

Add 10 μl "No membranes" to the cAMP standard (per well in duplicates) and the positive and negative controls

Add 10 μl "6 μg/well membranes" to GLP-1 and GLP-1 agonists (per well in duplicates/triplicates)

Pos. Control: 10 μl "no membranes"+10 μl AlphaScreen Buffer

Neg. Control: 10 μl "no membranes"+10 μl cAMP Stock Solution (50 μM)

As the beads are sensitive to direct light, any handling is in the dark (as dark as possible), or in green light. All dilutions are made on ice.

Procedure

1. Make the AlphaScreen Buffer.
2. Dissolve and dilute the GLP-1 agonists/cAMP standard in AlphaScreen Buffer.
3. Make the Donor Beads solution and incubate 30 min. at RT.
4. Add the cAMP/GLP-1 agonists to the plate: 10 μl per well.
5. Prepare membrane/Acceptor Beads solution and add this to the plates: 10 μl per well.
6. Add the Donor Beads: 30 μl per well.
7. Wrap the plate in aluminum foil and incubate on the shaker for 3 hours (very slowly) at RT.
8. Count on AlphaScreen—each plate pre incubates in the AlphaScreen for 3 minutes before counting.

The EC_{50} [pM] values may be calculated using the GraphPad Prism software (version 5). If desired, the fold variation in relation to GLP-1 may be calculated as EC_{50} (GLP-1)/EC_{50} (analogue)−3693.2.

Assay (II): Half-Life in Minipigs

The purpose of this study is to determine the protraction in vivo of GLP-1 agonists after i.v. administration to minipigs, i.e. the prolongation of their time of action. This is done in a pharmacokinetic (PK) study, where the terminal half-life of the GLP-1 agonist in question is determined. By

terminal half-life is generally meant the period of time it takes to halve a certain plasma concentration, measured after the initial distribution phase.

Male Göttingen minipigs are obtained from Ellegaard Göttingen Minipigs (Dalmose, Denmark) approximately 7-14 months of age and weighing from approximately 16-35 kg are used in the studies. The minipigs are housed individually and fed restrictedly once or twice daily with SDS minipig diet (Special Diets Services, Essex, UK). After at least 2 weeks of acclimatisation two permanent central venous catheters are implanted in vena cava caudalis or cranialis in each animal. The animals are allowed 1 week recovery after the surgery, and are then used for repeated pharmacokinetic studies with a suitable wash-out period between dosings.

The animals are fasted for approximately 18 h before dosing and for at least 4 h after dosing, but have ad libitum access to water during the whole period.

The GLP-1 agonist is dissolved in 50 mM sodium phosphate, 145 mM sodium chloride, 0.05% tween 80, pH 7.4 to a concentration of usually from 20-60 nmol/ml. Intravenous injections (the volume corresponding to usually 1-2 nmol/kg, for example 0.033 ml/kg) of the compounds are given through one catheter, and blood is sampled at predefined time points for up till 13 days post dosing (preferably through the other catheter). Blood samples (for example 0.8 ml) are collected in EDTA buffer (8 mM) and then centrifuged at 4° C. and 1942 G for 10 minutes. Plasma is pippetted into Micronic tubes on dry ice, and kept at −20° C. until analyzed for plasma concentration of the respective GLP-1 compound using ELISA or a similar antibody based assay or LC-MS. Individual plasma concentration-time profiles are analyzed by a non-compartmental model in WinNonlin v. 5.0 (Pharsight Inc., Mountain View, Calif., USA), and the resulting terminal half-lives (harmonic mean) determined.

Assay (III): Effect on Blood Glucose and Body Weight

The purpose of the study is to verify the effect of GLP-1 agonists on blood glucose (BG) and body weight (BW) in a diabetic setting. GLP-1 agonists may be tested in a dose-response study in an obese, diabetic mouse model (db/db mice) as described in the following.

Fifty db/db mice (Taconic, Denmark), fed from birth with the diet NIH31 (NIH 31M Rodent Diet, commercially available from Taconic Farms, Inc., US, see www.taconic-.com), are enrolled for the study at the age of 7-9 weeks The mice are given free access to standard chow (e.g. Altromin 1324, Brogaarden, Gentofte, Denmark) and tap water and kept at 24° C. After 1-2 weeks of acclimatisation, the basal blood glucose is assessed twice on two consecutive days (i.e. at 9 am). The 8 mice with the lowest blood glucose values may be excluded from the experiments. Based on the mean blood glucose values, the remaining 42 mice may be selected for further experimentation and allocated to 7 groups (n=6) with matching blood glucose levels. The mice may be used in experiments with duration of 5 days for up to 4 times. After the last experiment the mice are euthanised.

The seven groups may receive treatment as follows:

1: Vehicle, subcutaneous
2: GLP-1 agonist, 0.3 nmol/kg, subcutaneous
3: GLP-1 agonist, 1.0 nmol/kg, subcutaneous
4: GLP-1 agonist, 3.0 nmol/kg, subcutaneous
5: GLP-1 agonist, 10 nmol/kg, subcutaneous
6: GLP-1 agonist, 30 nmol/kg, subcutaneous
7: GLP-1 agonist, 100 nmol/kg, subcutaneous

Vehicle: 50 mM sodium phosphate, 145 mM sodium chloride, 0.05% tween 80, pH 7.4.

The GLP-1 agonist is dissolved in the vehicle, e.g. to concentrations of 0.05, 0.17, 0.5, 1.7, 5.0 and 17.0 nmol/ml. Animals are dosed subcutaneous with a dose-volume of 6 ml/kg (i.e. 300 µl per 50 g mouse).

On the day of dosing, blood glucose is assessed at time −½ h (8.30 am), where after the mice are weighed. The GLP-1 agonist is dosed at approximately 9 am (time 0). On the day of dosing, blood glucose is assessed e.g. at times 1, 2, 4 and 8 h (10 am, 11 am, 1 pm and 5 pm).

On the following days, the blood glucose is assessed e.g. at time 24, 48, 72, and 96 h after dosing (i.e. at 9 am on day 2, 3, 4, 5). On each day, the mice are weighed following blood glucose sampling.

The mice are weighed individually on a digital weight.

Samples for the measurement of blood glucose are obtained from the tail tip capillary of conscious mice. Blood, 10 µl, is collected into heparinised capillaries and transferred to 500 µl glucose buffer (EKF system solution, Eppendorf, Germany). The glucose concentration is measured using the glucose oxidase method (glucose analyser Biosen 5040, EKF Diagnostic, GmbH, Barleben, Germany). The samples are kept at room temperature for up to 1 h until analysis. If analysis has to be postponed, samples are kept at 4° C. for a maximum of 24 h.

ED_{50} is the dose giving rise to half-maximal effect in nmol/kg. This value is calculated on the basis of the ability of the GLP-1 agonists to lower body weight as well as the ability to lower blood glucose, as explained below.

ED_{50} for body weight is calculated as the dose giving rise to half-maximum effect on delta BW 24 hours following the subcutaneous administration of the GLP-1 agonist. For example, if the maximum decrease in body weight after 24 hours is 4.0 g, then ED_{50} bodyweight would be that dose in nmol/kg which gives rise to a decrease in body weight after 24 hours of 2.0 g. This dose (ED_{50} body weight) may be read from the dose-response curve.

ED_{50} for blood glucose is calculated as the dose giving rise to half-maximum effect on AUC delta BG 8 hours following the subcutaneous administration of the GLP-1 agonist.

The ED_{50} value may only be calculated if a proper sigmoidal dose-response relationship exists with a clear definition of the maximum response. Thus, if this would not be the case the GLP-1 agonist in question is re-tested in a different range of doses until the sigmoidal dose-response relationship is obtained.

Assay (IV): Effect on Food Intake

The purpose of this experiment is to investigate the effect of GLP-1 agonists on food intake in pigs. This is done in a pharmacodynamic (PD) study as described below, in which food intake is measured 1, 2, 3, and 4 days after administration of a single dose of the GLP-1 agonist, as compared to a vehicle-treated control group.

Female Landrace Yorkshire Duroc (LYD) pigs, approximately 3 months of age, weighing approximately 30-35 kg are used (n=3-4 per group). The animals are housed in a group for 1-2 weeks during acclimatisation to the animal facilities. During the experimental period the animals are placed in individual pens from Monday morning to Friday afternoon for measurement of individual food intake. The animals are fed ad libitum with pig fodder (Svinefoder, Antonio) at all times both during the acclimatisation and the experimental period. Food intake is monitored on line by logging the weight of fodder every 15 minutes. The system used is Mpigwin (Ellegaard Systems, Faaborg, Denmark).

The GLP-1 agonists are dissolved in a phosphate buffer (50 mM phosphate, 0.05% tween 80, pH 8) e.g. at concen-

trations of 12, 40, 120, 400 or 1200 nmol/ml corresponding to doses of 0.3, 1, 3, 10, or 30 nmol/kg. The phosphate buffer served as vehicle. Animals are dosed with a single subcutaneous dose of the GLP-1 agonist or vehicle (dose volume 0.025 ml/kg) on the morning of day 1, and food intake is measured for 4 days after dosing. On the last day of each study, 4 days after dosing, a blood sample for measurement of plasma exposure of the GLP-1 agonist is taken from the heart in anaesthetised animals. The animals are thereafter euthanised with an intra-cardial overdose of pentobarbitone. Plasma content of the GLP-1 agonist is analysed using ELISA or a similar antibody based assay.

Food intake is calculated as mean±SEM 24 h food intake on the 4 days. Statistical comparisons of the 24 hour food intake in the vehicle vs. GLP-1 agonist group on the 4 days are done using one-way or two-way-ANOVA repeated measures, followed by Bonferroni post-test.

Assay (V): Stability Against Degradation by Intestinal Enzymes

The purpose of this example is to test the stability against degradation by intestinal enzymes. GLP-1(7-37) may be used in the assay as a comparative compound. The strongest proteolytic activities in the intestine are of pancreatic origin and include the serine endopeptidases trypsin, chymotrypsin, and elastase as well as several types of carboxypeptidases. An assay with small intestine extract from rats was developed and used as described in the following.

Extracts from Rat Small Intestine

Small intestines are prepared from rats and flushed with 8 ml of 150 mM NaCl, 20 mM Hepes pH 7.4. The solutions are centrifuged for 15 min at 4,600 rpm in a Heraeus Multifuge 3 S-R centrifuge with a 75006445 rotor. The supernatants are removed and filtered through a 0.22 μm Millipore Millex GV PVDF membrane. Filtrates of several animals are pooled to average out individual differences.

The protein content of the obtained extracts is determined by Bradford Assay (see e.g. Analytical Biochemistry (1976), vol. 72, p. 248-254, and Analytical Biochemistry (1996), vol. 236 p. 302-308).

Degradation Assay

2.5 nmol of the GLP-1 agonists to be tested are incubated with the intestinal extract in a volume of 250 μl at 37° C. over a period of one hour. Intestinal samples are assayed in presence of 20 mM Hepes at pH 7.4. The concentration of the intestinal extract is titrated in pilot experiments so that the half-life (t½) of GLP-1(7-37) is in the range of 10-20 minutes. The small intestine extract is used at a concentration of 1.4 μg/ml. All components except for the intestinal extract are mixed and pre-warmed for ten minutes at 37° C. Immediately after addition of the intestinal extract a sample of 50 μl is taken and mixed with the same volume of 1% trifluoroacetic acid (TFA). Further samples are taken accordingly after 15, 30, and 60 minutes.

Sample Analysis

UPLC Analysis:

10 μl of the samples are analysed by UPLC using a Waters Acquity system with a BEH C18 1.7 μm 2.1×50 mm column and a 30 to 65% gradient of 0.1% TFA and 0.07% TFA in acetonitrile over 5 minutes at a flow rate of 0.6 ml/min. After baseline subtraction the peak integrals of the intact compounds in the HPLC chromatogram recorded at a wavelength of 214 nm are determined.

Maldi-TOF Analysis:

1 μl of each sample is transferred to a Bruker/Eppendorf PAC HCCA 384 MALDI target. Analysis is performed with a Bruker Autoflex matrix-assisted laser desorption and ionisation—time of flight (MALDI-TOF) mass spectrometer using the pre-defined method "PAC measure" with an extended detection range of 500 to 5000 Da and the predefined calibration method "PAC_calibrate".

Data Analysis:

The peak integrals of the HPLC chromatograms are plotted against time. The half-life of the respective compound is calculated by fitting the data using SigmaPlot 9.0 software and an equation for a 2-parameter exponential decay. For each GLP-1 agonist tested, the relative half-life (relative $T_{1/2}$) is calculated as the half-life ($T_{1/2}$) of the compound in question, divided by the half-life ($T_{1/2}$) of GLP-1(7-37), determined in the same way.

While certain features of the invention have been illustrated and described herein, many modifications, substitutions, changes, and equivalents will now occur to those of ordinary skill in the art. It is, therefore, to be understood that the appended claims are intended to cover all such modifications and changes as fall within the true spirit of the invention.

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The invention claimed is:

1. A method for treating type 2 diabetes, comprising administering semaglutide once weekly in an amount of 1.0 mg to a subject in need thereof.

2. The method according to claim **1**, wherein the semaglutide is administered by parenteral administration.

3. The method according to claim **2**, wherein the solution is administered by subcutaneous injection.

4. The method according to claim **1**, wherein the semaglutide is administered in the form of an isotonic aqueous solution comprising phosphate buffer at a pH in the range of 7.0-9.0.

5. The method according to claim **4**, wherein the solution further comprises propylene glycol and phenol.

6. The method according to claim **4**, wherein the pH is 7.4.

7. The method according to claim **6**, wherein the solution further comprises propylene glycol and phenol.

8. The method according to claim **4**, wherein the phosphate buffer is a sodium dihydrogen phosphate buffer.

9. The method according to claim **1**, wherein the semaglutide is administered by subcutaneous injection in the form of an isotonic aqueous solution comprising at a sodium dihydrogen phosphate buffer at a pH in the range of 7.0-9.0, and wherein the solution further comprises propylene glycol and phenol.

10. The method according to claim **9**, wherein the pH is 7.4.

* * * * *